

TITLE

STARCH WITH REDUCED AMYLOSE CONTENT

FIELD OF THE INVENTION

The present invention relates to food products, particularly pourable food products or bakery products (such as various breads) which exhibit a reduced tendency to retrograde or stale, respectively. The invention also relates to modified starch replacers. The invention also relates to methods of plant breeding and to various probes for use in plant breeding. The invention also relates to improved high ratio cake flours and to improved methods for preparing high ratio cake flours. In particular, the present invention relates to food products, methods and processes based on starches having particular qualities associated with the amylose content, amylose quality, amylose crystallisation properties, processing histories and/or botanic origin. The products are starch-based products ranging from pourable or spoonable food products or pastes to bakery products such as various breads and cakes.

IntroductionStarch

Starch is the major storage product of the world's most important food crops and is found in large quantities in the seeds of cereals (such as wheat, corn and rice), in legumes (such as pea) and in tuber and root crops such as potato and yam.

Starch is laid down in higher plants in the form of insoluble grains or granules that act as an energy reserve. The starch granule usually comprises two different polymers: amylose (an essentially linear chain of $\alpha(1-4)$ -linked α -D-glucopyranosyl residues) and amylopectin (which comprises highly branched $\alpha(1-4)$ -linked α -D-glucopyranosyl residues, the branching occurring via $\alpha(1-6)$ linkages).

Together, amylose and amylopectin make up 97-99% of the dry weight of starch. Other minor constituents include lipids (principally occurring in cereal starches), protein and trace elements (e.g., phosphorous).

Amylose

Two crystalline forms of amylose can be identified: the so-called "A" and "B" forms. The "B" form is formed during retrogradation at room temperature, while the "A" form can be grown under other conditions (for example, at temperatures above 50°C). The "A" form can also be produced with short chain amylose, which as used herein defines amylose having a molecular weight distribution sufficiently small such that "A" type crystals preferentially form. Both "A" and "B" forms are believed to form crystals based on regular parallel packing of amylose double helices, with the different forms having different unit

cells in which the packing leads to significant differences in the positioning of water (in the "A" crystals, four water molecules are located between the helices, as oppose to thirty six in the "B" form).

5 Amylose has very limited branching (about one branch point for every few thousand glucose units). Molecular weights are typically around 10^5 - 10^6 . Amylopectin is much more branched than amylose, with typically 5% of the glucose units containing $\alpha(1-6)$ -linked branch points to connect the $\alpha(1-4)$ -linked chains. The molecular weight is much higher than amylose, typically in excess of 10^8 .

10 The amylose contents of different starches varies. Potato and tapioca starch typically have much lower amylose contents (21% and 17%, respectively) than the 28% found in maize and wheat starch.

Amylose can complex with lipid, wherein the lipid molecule resides within a single helix of amylose. Such complexes are termed helical inclusion
15 complexes, and it has now been found that they can have an important influence on amylose crystallisation behaviour, retrogradation and staling.

Different morphic forms of amylose also exist, as described *infra*.

Starch granule structure

20 Starch granules vary in size and shape with botanic origin. Potato starch granules for example are large (up to 100 μm in diameter) and oval, whereas rice starch granules are smaller (at most 10 μm in diameter), and much more angular. Wheat and barley starch granules have a bimodal size distribution: large A-type granules (diameter about 10-30 μm) and smaller B-type granules (diameter about 5 μm). All starch granules have a complex structure, containing structural order
25 on more than one level.

Examination of starch grains by optical or electron microscopy reveals pronounced concentric rings called "growth rings" (see Fig. 1). These rings are alternately of semi-crystalline and amorphous composition, and vary from between 1200-7000 Å. The relative sizes of the growth rings can be altered by
30 growth conditions, and in particular the lighting conditions. In some species, growth rings can be eliminated by growing under constant light conditions.

The semi-crystalline rings are composed of stacks of alternating crystalline and amorphous lamellae (see also Fig. 1). These lamellae arise from a radial arrangement of clusters of amylopectin, which clusters contain regions high in
35 branch points (the amorphous lamellae) and a region where short chain segments of amylopectin have formed double helices (the crystalline lamellae - see e.g., Kainuma and French (1972), Biopolymers, 11:2241-2250).

The size of the clusters appears to be constant for all botanic sources, mutant and wild type starches (about 90 Å), but within this cluster the relative

extent of the amorphous lamellae and the crystalline lamellae may vary. For example, waxy and partial waxy mutant starches exhibit relatively thick amorphous lamellae and/or relatively thin crystalline lamellae, while the presence of amylose increases the relative thickness of the crystalline lamellae (see Fig. 2).

5 Mutant starches

Alongside the vast number of naturally occurring starches, plant breeders have applied various forms of selective breeding techniques and genetic manipulation to generate a range of mutant starches.

For example, mutations or genetic modifications that eliminate the activity
10 of the amylose synthetic enzyme(s) (often collectively referred to as granule bound starch synthase, GBSS or the Wx protein(s)) result in amylose-free (waxy) starch. In such mutants, the starch granule contains only amylopectin. The mutants (and the starches obtained therefrom) are known as waxy mutants and waxy starches, respectively. In diploid grasses (such as barley and maize), many
15 different waxy mutants have been found. Waxy mutants have also been identified in rice and potato.

In polyploid plants, however, a plurality of different GBSS (Wx protein) isoenzymes may contribute to amylose synthesis. Common wheat, for example, is allohexaploid (AABBDD) and there are three loci (*Wx-A1*, *Wx-B1* and *Wx-D1*)
20 encoding three isoforms of the Wx protein (GBSS), one on each of the three chromosomes. A spontaneous waxy mutant not producing amylose would require simultaneous recessive mutations in all three loci (an extremely unlikely event) and such wheat varieties have not yet been identified (though waxy wheats have been produced *via* hybridizations of lines carrying null (non-functional) alleles at
25 the *Wx* loci of each of the A, B and D genomes).

The three GBSS (Wx) isoforms in wheat can be separated into high molecular weight (HMW) and low molecular weight (LMW) proteins, the HMW Wx protein being encoded by the A genome and a mixture of LMW Wx proteins by the B and D genomes (Nakamura *et al.* (1992), Japan. J. Breed. 42:681-685).

30 The various GBSS enzymes may exhibit differential sensitivity to inhibitors, stability, half-life *in vivo*, specific activity and/or affinity for substrate(s) or effector molecules. They may act in different cellular compartments, for example being localized in or on the starch granule, in the soluble phase or at the interface between granule and soluble phase. They may
35 have different side activities and be associated with different multienzyme complexes. They may exhibit differential interactions with the starch granule, temperature sensitivities and responses to grain water content. They may also contain different effector binding sites.

Any or all of these different structural, chemical and/or biochemical properties may be associated with differences in the quantity and quality of the cognate amylose product, so giving rise to different morphic forms of amylose. The different morphic forms of amylose may differ *inter alia* with respect to

5 molecular weight (or degree of polymerisation), degree and/or extent of branching, associative activity or affinity with respect to other non starch polysaccharides (such as pentosans) and site of synthesis *in vivo*. These qualitatively different forms of amylose are referred to herein as *morphic forms* (i.e., they do not correspond to the polymorphic crystalline forms (the "A" and

10 "B" forms) described earlier, but rather represent an entirely different level of complexity).

Wheat varieties have been identified in which two of the three Wx proteins are eliminated, with the result that amylose content is significantly reduced (to about 20%). In some of these cultivars, the *Wx-A1* and *Wx-B1* loci are inactive,

15 only the *Wx-D1* locus being active. These cultivars have been designated "partial waxy mutants", and the starches isolated therefrom "partial waxy mutant starches" (see Nakamura *et al.*, (1993) Plant Breeding; 111:99-105).

As used herein the term partial waxy mutant starch is used in a somewhat broader sense to embrace starches derived from any plant source (including

20 wheat) in which either: (a) the amylose content of the starch granule is reduced (but not entirely eliminated) as a result of mutation of one or more of the *waxy* (*Wx*) loci or genes; and/or (b) one or more *Wx* null alleles (e.g., *Wx-A1b*, *Wx-B1b* and/or *Wx-D1b*) are present, together with at least one functional *Wx* gene; and/or (c) double null *Wx* alleles are present.

Thus, some partial waxy mutant starches may contain lower amylose contents relative to wild-type counterparts. However, in some partial waxy mutant starches (particularly those derived from single rather than double null lines) the levels of amylose may be unaltered, for example as a result of dosage compensation by the remaining active *Wx* gene(s). In the latter case, the amylose

30 (and/or the distribution/profile of amylose morphic forms) may differ qualitatively (rather than merely quantitatively) from that present in wild type starches.

Such mutant starches may arise when the amount of Wx protein is reduced (but not eliminated), for example *via* mutation of one or more *Wx* loci or structural genes. In most cases, partial waxy mutant starches are those produced by cultivars

35 in which one or more (but not all) of a plurality of Wx protein isoforms is inactive or absent. In the case of wheat, partial waxy mutant starches may be those derived from cultivars in which one or two of the three Wx isoforms (*Wx-A1*, *Wx-B1* and *Wx-D1*) are inactive or absent.

The term "mutant starch", as used herein, is intended to define any starch which has been produced from a plant source which has been manipulated in such a way so as to alter the characteristics of the starch produced by the plant. Such manipulation may be effected by any genetic manipulation or breeding technique.

- 5 For example, genetic manipulation may include mutagenesis (including site-directed mutagenesis), insertional mutagenesis, gene deletion or substitution, transposon mutagenesis, the introduction of frameshift mutations or the use of antisense DNA technology to attenuate (or eliminate) mRNA translation.

- 10 Other techniques include those based on environmental (non-genetic) manipulation (for example, the administration of growth regulating compositions, the regulation of water availability, temperature and/or lighting conditions during growth of the source plant).

The term "mutant starch granule" is to be interpreted *mutatis mutandis*.

Starch gelatinisation

- 15 When heated in the presence of water, starch undergoes an irreversible order-disorder transition termed gelatinisation. The granules are observed to swell, absorb water, lose crystallinity and leach amylose. The swelling of the amorphous regions of the granule strips starch chains from the surface of the crystalline regions, thereby disrupting crystalline order. The stripping process
20 results in unfolding and hydration of helices in the amylose and amylopectin constituents.

- The gelatinisation process is accompanied by an endotherm which can be measured by differential scanning calorimetry, important parameters of which are the gelatinisation enthalpy and the gelatinisation endotherm width (see e.g.,
25 Donovan (1979), Biopolymers, 18:263-275).

As used herein, the term "gelatinised" (as applied to starch) is intended to define an amylose/amylopectin complex which is *disordered* relative to the ordered state of these components in the starch granule. The term "gelatinisation" is to be interpreted accordingly.

- 30 As used herein, these terms therefore embraces states in which gelatinisation is incomplete (as may occur for example in various extrusion and steam treatments) and/or has proceeded in the presence of limiting (rather than excess) water.

Starch retrogradation

- 35 Native starch has a semi-crystalline structure which is partially or completely lost during gelatinisation. During subsequent ageing, the gelatinised material regains some order, a process known as *retrogradation*. This process is characterized by the slow formation of "B" form amylose crystals with time, which is correlated with an increase in shear modulus on storage. The

development of crystallinity on ageing is also accompanied by an endothermic transition at 50-60°C, which arises from amylopectin crystallisation. While the amylopectin crystallisation is reversible, that of amylose is not.

5 Retrogradation is associated with the staling ("firming") of bread and setting of starch-based food thickeners, soups, gravies, sauces and similar products. It also leads to phase separation, syneresis and/or structural breakdown of starch-based creams, jellies and dressings. In many circumstances, it is a major problem in the food industry.

10 In traditional cookery, unmodified flours and starches (e.g., wheat flour, cornflour) are used to thicken sauces, soups etc. On cooking in aqueous liquids, the starch component gelatinises and swells to produce a thickening effect. Foodstuffs made in this way exhibit very acceptable eating quality when used fresh. However, when cooled for storage the starch retrogrades to form a solid matrix, and the foodstuff sets. This can be partially reversed by heating before
15 consumption, but the eating quality of the freshly-prepared product is not completely restored.

Such behaviour, although acceptable for many domestic situations, is not satisfactory for most industrially-prepared foodstuffs. In such cases, ambient- or chill-stable products are required generally to remain fluid and not to exhibit
20 typical retrogradation characteristics.

This problem has been addressed by the use of flours and starches which do not contain any amylose; so-called "waxy" cereals. In diploid grasses (such as barley and maize) mutations which eliminate GBSS activity (so producing amylose-free starch) are common and well characterized, and there is considerable
25 interest in the production of waxy wheat mutants (Nakamura *et al.* (1995), *Mol Gen Genet*; 248,253). Such mutant wheat varieties are expected to find utility in all present applications of waxy maize starch. However, such materials have an undesirable long, stringy texture. Thus, they are usually chemically modified, derivitised or substituted (acetylated etc.), and although this yields the required
30 functionality such starches must be declared as additives in many countries ("chemically modified starch") and a significant number of consumers would prefer to see a natural alternative. Such chemically modified starches are referred to herein as modified starches.

It has now been unexpectedly discovered that *partial* waxy starches (and
35 not, as has generally been assumed, *fully* waxy starches) have particular advantages in certain food applications where retrogradation characteristics must be controlled. In particular, it has been found that the use of partial waxy wheat starch may be used to produce non-retrograding food products which do not exhibit the disadvantageous organoleptic and textural properties of products based

on waxy starches (e.g. they may be non-stringy, non-mucilaginous and have a low degree of elasticity).

As used herein, the term "non-retrograding" is intended to define products containing gelatinised starch in which the rate of retrogradation is substantially reduced, for example to a level such that the products do not set (i.e., they remain pourable or substantially fluid) after storage (for example after cooled storage, e.g., at about 1-10°C) for one or more days. Typically, the liquid or pourable non-retrograding products of the invention exhibit substantially no increase in viscosity over a 30 min period after gelatinisation at 5°C.

As used herein, the term "non staling" is intended to define products containing gelatinised starch in which staling has been retarded, delayed or substantially eliminated.

SUMMARY OF THE INVENTION

According to the present invention there is provided a non-retrograding food product comprising gelatinised starch, wherein the starch:

- (a) comprises (or consists of) a partial waxy mutant starch; and/or
 - (b) has an amylose content of less than 30%, for example an amylose content of less than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28% or 29%;
- the food product for example being pourable, liquid or spoonable.

In another aspect, there is provided a non-retrograding food product (for example as defined in Claim 1) comprising gelatinised starch, wherein the starch:

- (a) is derived from a mutant starch granule having crystalline and amorphous lamellae in which the thickness of the amorphous lamellae is increased relative to that in the wild type starch; and/or
- (b) has a gelatinisation enthalpy of about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 J/g, as measured by DSC of a 40% w/w starch solution; and/or
- (c) has a gelatinisation endotherm width of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40°C, as measured by DSC of a 40% w/w starch solution; and/or
- (d) is a mutant starch having a high gelatinisation enthalpy relative to that of the wild type starch; and/or
- (e) is a mutant starch having a narrow gelatinisation endotherm width relative to the wild type starch; and/or
- (f) is derived from a mutant starch granule having a low bulk density relative to the wild type starch granule.

Also provided is a non-retrograding food product (for example as defined above) comprising gelatinised starch, wherein the starch amylose:

- (a) is associated with lipid such that greater than 20% w/w (e.g., greater than 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% w/w) is associated therewith; and/or
- (b) is mono-, di- or oligomeric, for example such that:
- (i) the amylose exhibits a reduced tendency to retrograde; and/or
- (ii) the bulk density of the starch granule is decreased relative to the corresponding polymorphic form; and/or
- (iii) the gelatinisation enthalpy of the starch is as defined above; and/or
- (iv) the gelatinisation endotherm width of the starch is as defined above; and/or
- (c) forms futile and/or competing associations with non-starch polysaccharides (e.g., pentosans) in the food product which decrease its tendency to crystallise (e.g., into the "B" form) such that the food product is non-retrograding; and/or
- (d) does not contain significant amounts of Amy^B; and/or
- (e) consists of any one of Amy^A, Amy^B or Amy^D; and/or
- (f) consists of any two of Amy^A, Amy^B or Amy^D; and/or
- (g) consists of Amy^D or a combination of Amy^A and Amy^D; and/or
- (h) comprises or consists of a morphic form having a peak degree of polymerization of:
- (i) 500-1000 (e.g., about 750); or
- (ii) 1500-2500 (e.g., about 2000); or
- (iii) 2700-3200 (e.g., about 3000); or
- (iv) 4500-5500 (e.g., about 5000); or
- (v) 5500-6000 (e.g., about 5700); or
- (vi) 6000-6500; or
- (vii) 6500-7000; or
- (viii) 7000-7500; or
- (ix) 7500-8000; or
- (x) 8000-8500; or
- (xi) 8500-9000; or
- (xii) 9000-9500; or
- (xiii) 9500-10000; or
- (xiv) 10000-11000; or
- (xv) 11000-12000; or

- (xvi) 12000-13000, or
(xvii) 13000-14000, or
(xviii) 14000-15000, or
(xix) 15000-17000; and/or
- 5 (i) comprises or consists of a morphic form which is branched such that it has an average number of:
- (i) less than 2 chains; or
(ii) less than 3 chains; or
(iii) less than 4 chains; or
10 (iv) less than 5 chains; or
(v) less than 6 chains; or
(vi) less than 7 chains; or
(vii) less than 8 chains; or
(viii) less than 9 chains; or
15 (ix) less than 10 chains; and/or
- (j) comprises or consists of a morphic form having a molar fraction of branched amylose of:
- (i) less than 5%; or
(ii) less than 10%; or
20 (iii) less than 15%; or
(iv) less than 20%; or
(v) less than 25%; or
(vi) less than 30%; or
(vii) less than 35%; or
25 (viii) less than 40%; or
(ix) less than 45%; or
(x) less than 50%; or
(xi) less than 55%; and/or
- (k) comprises or consists of a morphic form having a degree of
30 polymerisation and/or branching such that:
- (i) it crystallises to form essentially monomorphic (e.g., A form) crystals; and/or
(ii) A form crystals are preferentially produced during incubation at 5°C and/or
35 (iii) the ratio of A form to B form crystals produced during crystallisation is such that retrogradation is inhibited or abolished; and/or
- (l) is derived from a mutant starch in which the stoichiometry of the different morphic forms of amylose (e.g., Amy^A, Amy^B and/or Amy^D, or their species variants or homologues) is perturbed; and/or

- (m) is derived from a mutant starch in which the amount of amylose associated with lipid is increased relative to the wild type starch; and/or
- (n) is derived from a mutant starch in which the number of different morphic forms of amylose is reduced relative to the wild type starch (for example to one or two morphic forms); and/or
- (o) is derived from a mutant starch in which the amylose has a higher affinity for non starch polysaccharides (e.g., pentosans) relative to the wild type starch; and/or
- (p) is derived from a mutant starch in which the degree of polymerization of the amylose is low relative to the wild type starch; and/or
- (q) is derived from a mutant starch in which the degree of branching is low relative to the wild type starch; and/or
- (r) is derived from a mutant starch in which the degree of branching is high relative to the wild type starch; and/or
- (s) is derived from a mutant starch in which the molar fraction of branched amylose is high relative to the wild type starch; and/or
- (t) is derived from a mutant starch in which the molar fraction of branched amylose is low relative to the wild type starch; and/or
- (u) is derived from a mutant starch in which the amylose forms more A type crystals (or less B type crystals) relative to the wild type starch; and/or
- (v) comprises (or consists of) short chain amylose; and/or
- (w) comprises (or consists of) amylose which forms helical inclusion complexes with lipids (e.g., monoglycerides) to an extent sufficient to render the amylose non-retrograding.
- Also provided is a non-retrograding food product (for example as defined above) comprising gelatinised starch, wherein the starch is derived from:
- (a) a plant source having partially depressed amylose synthase activity; and/or
- (b) a *Wx* mutant plant source; and/or
- (c) a plant source having a *Wx* null allele; and/or
- (d) a plant source having *Wx* double null alleles; and/or
- (e) a plant source having a modified GBSS isoenzyme activity profile; and/or
- (f) a plant source which is a polyploid (e.g., tetraploid or hexaploid) plant variety in which the expression of the *Wx-B1* gene (or a species variant/homologue thereof) is reduced or eliminated and/or the expression of the *Wx-D1* gene (or a species variant/homologue thereof) is increased relative to that of the *Wx* gene(s) on the other chromosome(s); and/or

(g) a mutant plant source having a reduced range of starch grain morphologies (the starch grains for example being monodisperse or oligodisperse); and/or

(h) a partial waxy mutant plant source; and/or

5 (i) a mutant plant source bearing a mutation in a *Wx* regulatory protein associated with the B genome of wheat (or species variants/homologues thereof).

Preferably, the activity of one or more GBSS isoenzyme(s) in the plant source is decreased or eliminated. Alternatively, the activity of two or more GBSS isoenzymes may be decreased or eliminated. Thus, only one GBSS
10 isoenzyme may be active or expressed, and preferably, only two GBSS isoenzymes are active or expressed.

Also provided is a non-retrograding food product (for example as defined above) comprising gelatinised starch, wherein the starch has been subjected to a physical (e.g., shear, ultrasound or ionising radiation), biochemical (e.g., enzymic,
15 e.g. amylolytic) and/or chemical (e.g., hydrolysis or chemical spiking, for example with non-starch polysaccharides (e.g., pentosans), lipids (e.g., monoglycerides) or proteins) treatment which:

(a) is sufficient to render it non-retrograding; and/or

(b) facilitates non-productive association between non starch
20 polysaccharides (e.g., pentosans) and starch-derived amylose such that amylose crystallisation (e.g., into the "B" form) is reduced or eliminated; and/or

(c) is sufficient to reduce the chain length of the amylose to an extent sufficient to render it non-retrograding and/or to an extent sufficient to facilitate non-productive association between non starch polysaccharides (e.g., pentosans)
25 and amylose such that amylose crystallisation (e.g., into the "B" form) is reduced or eliminated and/or to an extent to reduce or eliminate the ability of the amylose to crystallise after gelatinisation.

The starch may be derived from a hexaploid wheat variety in which the expression of the gene encoding GBSS on:

30 (a) the A genome; or

(b) the B genome; or

(c) the D genome; or

(d) the A and B genomes; or

(e) the A and D genomes; or

35 (f) the B and D genomes,

is reduced or eliminated.

Preferably, the expression of the gene encoding GBSS on the B genome is reduced or eliminated.

In preferred embodiments, the starch is derived from a hexaploid wheat variety in which the expression of the gene encoding GBSS on the D genome is increased relative to that of the gene(s) on the other chromosome(s).

In another aspect, the invention provides a non-retrograding food product
5 (for example as defined above), which food product comprises gelatinised starch wherein the amylose is di- or oligomorphous, each morphic form: (a) having a peak degree of polymerization selected from the values set out above, and/or (b) being branched such that it has an average number of chains selected from the values set out above, and/or (c) having a molar fraction of branched amylose selected from
10 the values set out above.

Also provided is a non-retrograding food product (for example as defined above), which food product comprises gelatinised starch wherein the amylose is di- or oligomorphous, the peak degree of polymerization and/or degree of branching and/or molar fraction of branched molecules being substantially homogeneous,
15 similar or identical.

The food product is preferably:

- (a) a babyfood; or
- (b) a bakery product (for example a bread, yeasted goods or a cake); or
- (c) a bakery supply product (for example, a custard or a bakery filling
20 or topping); or
- (d) a batter; or
- (e) a breadding; or
- (f) a cereal; or
- (g) a confectionary; or
- (h) a flavour or beverage emulsion; or
- (i) a fruit filling; or
- (j) a gravy, soup, sauce or food thickener;
- (k) a frozen, chilled or ambient stable gravy, soup, sauce or food
thickener; or
- (l) a pasteurised, retorted or UHT treated gravy, soup, sauce or food
30 thickener; or
- (m) a meal or meal component; or
- (n) a meat product; or
- (o) a petfood; or
- (p) a pharmaceutical or nutraceutical; or
- (q) a potato product; or
- (r) a dairy product (e.g. a dessert or yogurt); or
- (s) a salad dressing; or
- (t) a snack or cracker; or

(u) a spread; or

(v) a pasta product (e.g. a noodle).

The plant source may be a cereal (e.g., wheat, rice, barley or corn), legume (e.g., pea), tuber or root (e.g., potato).

5 In another aspect, the invention provides a process for producing a non-retrograding food product (for example as defined above) comprising the steps of:

(a) screening plant lines for a desired genotype, phenotype, starch or starch grain type having characteristics as defined in any one of the preceding claims and selecting a plant line having the desired characteristics; or

10 (a') mutagenising a plant (e.g., by insertional mutagenesis or antisense DNA technology) to produce a desired genotype yielding a starch or starch grain type having characteristics as defined in any one of the preceding claims; and then

(b) formulating a non-retrograding food product comprising starch extracted from plants bred from the plant line selected in step (a) or step (a').

15 The invention also contemplates a food product obtainable by (or produced by) such a process (for example being as defined hereinabove).

Also contemplated is a process for producing a high ratio cake flour comprising heating a flour, wherein the flour comprises starch which is ungelatinised but otherwise as defined herein.

20 Also contemplated is a process for producing a high ratio cake flour comprising treating flour with chlorine gas, wherein the flour comprises starch which is ungelatinised but otherwise as defined hereinabove.

Also contemplated is a high ratio cake flour obtainable by (or produced by) the process of the invention.

25 In another aspect, the invention contemplates the use of flour comprising starch which is ungelatinised (but otherwise as defined above) in the preparation of a high ratio cake flour mix.

In another aspect, the invention contemplates a high ratio cake flour mix comprising starch which is ungelatinised but otherwise as defined above.

30 In another aspect, the invention contemplates the use of a composition (e.g., a flour) comprising ungelatinised starch as defined above as a modified starch replacement, or a modified starch replacer comprising ungelatinized starch as defined herein.

Also contemplated is a non-staling food product (e.g., bread, bun, burger bun, sandwich) comprising starch as defined herein.

Also contemplated is a method for producing a food product comprising the step of using starch as defined herein as a modified starch replacer.

Also contemplated is a method of plant (for example cereal, e.g., wheat or legume, e.g., pea) breeding, comprising the step of screening plants for a desired

genotype, phenotype, starch or starch grain type having characteristics as defined herein.

In another aspect, the invention relates to a nucleotide or antibody (e.g., monoclonal antibody) probe for use in the method described above.

5 In another aspect, the invention relates to a nucleotide probe (e.g., as defined above) for use in determining the *Wx* genotype of a plant (for example a cereal, e.g., wheat or a legume, e.g., pea).

In another aspect, the invention relates to a nucleotide probe (e.g., as defined above) specific for *Wx-A1*, *Wx-B1* or *Wx-D1* (or for species
10 variant/homologues thereof).

The invention also contemplates an antibody (for example a monoclonal antibody) specific for *Wx-A1*, *Wx-B1* or *Wx-D1* proteins (or a species variants/homologues thereof), for example for use in the method of the invention.

Also contemplated is a nucleotide or antibody (e.g., monoclonal antibody)
15 probe for use in determining the GBSS profile of a plant (for example a cereal, e.g., wheat, or a legume, e.g., pea).

In another aspect, the invention relates to a method of plant (for example cereal, e.g., wheat or legume, e.g., pea) breeding, comprising the step of screening plants by probing with an antibody or nucleotide probe as defined herein.

20 The invention also contemplates a freeze-thaw stable food product comprising starch as defined herein.

In another aspect, the invention relates to a method of thickening a food product comprising the step of:

(a) adding a composition (e.g., flour) comprising ungelatinised starch
25 which is otherwise as defined herein;

(b) gelatinising the starch to thicken the food product.

Also covered is a thickened food product obtainable by (or produced by) the aforementioned method.

The invention also contemplated a method for retarding staling of a food
30 product (e.g., a baked food product, such as bread, e.g., a burger bun or sandwich) comprising the step of formulating the food product with starch as defined herein.

The invention also contemplated a method for retarding retrogradation of a food product comprising the step of formulating the food product with starch as
hereinbefore defined.

35 Also contemplated is a dehydrated food base comprising ungelatinised starch which is otherwise as hereinbefore defined.

In another aspect, the invention relates to an instantisable food base (for example, a roux e.g., a roux blanc) comprising ungelatinised starch which is otherwise as hereinbefore defined.

DESCRIPTION OF THE INVENTION

According to the present invention there is provided a non-retrograding pourable food product comprising gelatinised starch, wherein the starch:

- 5 (a) comprises (or consists of) a partial waxy mutant starch; and/or (b) has an amylose content of less than 30%, for example an amylose content of less than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28% or 29%.

10 Preferably, the amylose content is sufficiently low such that the starch gelatinizes to form an amylopectin-continuous (rather than an amylose-continuous) system.

The starch of the invention preferably is provided as part of a flour, flour fraction or flour extract.

15 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a mutant starch granule having crystalline and amorphous lamellae in which the thickness of the amorphous layer is increased relative to that in the wild type starch.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch has a gelatinisation enthalpy of about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,
20 24 or 25 J/g, as measured by DSC of a 40% w/w starch solution.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch has a gelatinisation endotherm width of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40°C, as
25 measured by DSC of a 40% w/w starch solution.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is a mutant starch having a high gelatinisation enthalpy relative to that of the wild type starch.

30 An increase in gelatinisation enthalpy is associated with a decrease in the amount of amylose in the starch granule.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is a mutant starch having a low gelatinisation endotherm width relative to that of the wild type starch.

35 A lowering of the gelatinisation endotherm width is associated with a decreased level of amylose in the starch granule.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a mutant starch granule having a low bulk density relative to the wild type starch granule.

Without wishing to be bound by any theory, it is thought that efficient packing of the amylose and amylopectin in the starch grain (resulting in relatively high bulk densities) is dependent, at least in part, on the availability for packing of polydisperse amylose morphic forms. However, it has now been recognized that efficient packing in the ungelatinised starch granule (which leads to relatively high granule bulk densities) is correlated with efficient amylose crystallisation after gelatinisation (which produces retrogradation).

Thus, mutant starches with starch granules in which the amylose/amylopectin packing is relatively "loose" (leading to lower bulk densities at the level of the starch crystal) have improved retrogradation properties after gelatinisation. Such starch granules also have improved swelling characteristics useful e.g., in certain high ratio cake flours and in other applications (such as noodle manufacture).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is associated with lipid such that greater than 20% w/w (e.g. greater than 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49% or 50% w/w) is associated therewith.

Amylose associated with lipid does not efficiently crystallise after gelatinisation, and so starches containing such amylose exhibits improved retrogradation properties.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is mono-, di- or oligomorphous such that: (i) the amylose exhibits a reduced tendency to retrograde; and/or (ii) the bulk density of the starch granule is decreased relative to the corresponding polymorphic form; and/or (iii) the gelatinisation enthalpy of the starch is as defined above; and/or (iv) the gelatinisation endotherm width of the starch is as defined above.

The term "monomorphic", as applied to amylose, is intended to denote a class of amylose molecules which substantially share physicochemical attributes selected from for example molecular weight, branching characteristics, associative affinities for non starch polysaccharides (such as pentosans) and crystallisation properties.

Alternatively, or in addition, the term "monomorphic" may also denote a class of amylose molecules synthesised by a common GBSS isoenzyme (e.g. that encoded by the *Wx* gene corresponding to that on the A, B or D chromosome in wheat, e.g., the species variants or homologues which may be located on other

chromosomes in other botanic sources). Such amylose morphic forms are herein denoted Amy^A, Amy^B and Amy^D, respectively.

As used herein, the term "homologue", as applied to a gene or protein, is intended to define genes or proteins which are evolutionary related. Evolutionary relationships may be determined by sequence (either protein or nucleic acid sequence) comparisons. In general, related genes and proteins will exhibit a high degree of sequence similarity (e.g. greater than 50%, 60%, 70%, 80%, 90% or 95% sequence similarity). In some cases, homologous genes will specifically cross hybridize and may be identified by hybridization assay under selective (or stringent) hybridization conditions. Homologous proteins may be immunologically cross-reactive.

The term "species variant" is intended to define that counterpart to any given gene or protein occurring in one organism in another organism. Such species variants may be identified by sequence and/or functional similarities. They may for example be identified by nucleic acid hybridization assays (where species variants may specifically cross-hybridize) and/or immunological assays (where species variants of any given protein may cross react with an antibody raised against one of the species variants). Preferably, species variants exhibit greater than 50%, 60%, 70%, 80%, 90% or 95% sequence similarity.

The term "dimorphic" and "oligomorphic" are intended to be interpreted *mutatis mutandis* to denote amylose populations which contain two or a limited range of amylose morphic forms (relative to those present in a retrograding starch), respectively.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose forms futile and/or competing associations with non-starch polysaccharides (e.g. pentosans) which decrease its tendency to crystallise (e.g., into the "B" form) such that the food product is non-retrograding. The non-starch polysaccharides may be derived from the food or associated with the starch source (e.g., present in the flour).

The futile and/or competing interactions may arise without the need for prior treatment of the food product or the gelatinised starch. However, in some cases physical, chemical and/or biochemical pretreatments are required to facilitate the interactions. For example, it may be necessary to subject the gelatinised starch to shearing forces or other treatments (see *infra*).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is monomorphic, dimorphic or oligomorphic (as herein defined).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch consists of any one of Amy^A, Amy^B or Amy^D (as herein defined).

5 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose consists of any two of Amy^A, Amy^B or Amy^D (as herein defined).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch consists of Amy^D or a combination of Amy^A and Amy^D (as hereinbefore defined).

10 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose has a peak degree of polymerization of: (i) 500-1000 (e.g., about 750); or (ii) 1500-2500 (e.g., about 2000); or (iii) 2700-3200 (e.g., about 3000); or (iv) 4500-5500 (e.g., about 5000); or (v) 5500-6000 (e.g., about 5700); or (vi) 6000-6500; or
15 (vii) 6500-7000; or (viii) 7000-7500; or (ix) 7500-8000; or (x) 8000-8500; or (xi) 8500-9000; or (xii) 9000-9500; or (xiii) 9500-10000; or (xiv) 10000-11000; or (xv) 11000-12000, or (xvi) 12000-13000, or (xvii) 13000-14000, or (xviii) 14000-15000, or (xix) 15000-17000.

20 The peak degree of polymerization is that degree of polymerization which occurs at greatest frequency in a given population of morphic forms.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is branched such that it has an average number of: (i) less than 2 chains; or (ii) less than 3 chains; or (iii) less than 4 chains; or (iv) less than 5 chains; or (v) less than
25 6 chains; or (vi) less than 7 chains; or (vii) less than 8 chains; or (viii) less than 9 chains; or (ix) less than 10 chains.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose has a molar fraction of branched amylose of: (i) less than 5%; or (ii) less than 10%; or
30 (iii) less than 15%; or (iv) less than 20%; or (v) less than 25%; or (vi) less than 30%; or (vii) less than 35%; or (viii) less than 40%; or (ix) less than 45%; or (x) less than 50%; or (xi) less than 55%.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose has a degree of
35 polymerisation and/or branching such that: (i) it crystallises to form essentially monomorphous (e.g., A form) crystals; and/or (ii) A form crystals are preferentially produced during incubation at 5°C and/or (iii) the ratio of A form to B form crystals produced during crystallisation is such that retrogradation is inhibited or abolished.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the stoichiometry of the different morphic forms of amylose (e.g., Amy^A, Amy^B and/or Amy^D) is perturbed.

5 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the amount of amylose associated with lipid is increased relative to the wild type starch.

10 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the number of different morphic forms of amylose is reduced relative to the wild type starch (for example to one or two morphic forms).

15 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the amylose has a higher affinity for non starch polysaccharides (e.g., pentosans) relative to the wild type starch. The term "pentosan" is a term of art which includes arabinoxylans and heteroxylans.

20 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the degree of polymerization of the amylose is low relative to the wild type starch.

25 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a mutant starch in which the degree of branching is low relative to the wild type starch.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the degree of branching is high relative to the wild type starch.

30 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a mutant starch in which the molar fraction of branched amylose is high relative to the wild type starch.

35 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from a mutant starch in which the molar fraction of branched amylose is low relative to the wild type starch.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch amylose is derived from

a mutant starch in which the amylose forms more A type crystals (or less B type crystals) relative to the wild type starch.

5 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a plant source having partially depressed amylose synthase activity.

The amylose synthase activity may be that arising from GBSS. It may be the net amylose synthase activity, or that of one or more isoenzymes. In some cases, the net amylose synthase activity may be unaltered, but the plant source may still have a partially depressed amylose synthase activity if suppression or
10 inactivation of one isoenzyme induces a compensatory increase in the activity of a different isoenzyme.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a *Wx* mutant plant source.

15 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a plant source having a *Wx* null allele.

The term "null allele" as applied to a *Wx* locus or gene is intended to define a mutation which directly or indirectly blocks or attenuates the expression of a *Wx* gene (e.g. at the transcriptional, translational or posttranscriptional level).
20 Such null alleles may therefore lie within any of the *Wx* genes (e.g. the coding or regulatory regions thereof) or lie within regulatory genes which directly or indirectly control the expression of *Wx* genes. In preferred embodiments, the null alleles define mutation(s) in the *Wx* structural genes which give rise to a non-
25 functional gene product(s) (for example, deletion mutation(s) and/or frameshift mutation(s)).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a plant source having *Wx* double null alleles.

30 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a plant source having a modified GBSS isoenzyme activity profile. The isoenzyme profile is the relative activity of each of a plurality of different GBSS enzymes (e.g., the relative activity of the *WxA*-1, *WxB*-1 and *WxD*-1 proteins or their
35 species variants/homologues in any given botanic source).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a plant source which is a polyploid (e.g., tetraploid or hexaploid) plant variety in which the expression of the *Wx-B1* gene is reduced or eliminated and/or the expression

of the *Wx-D1* gene is increased relative to that of the *Wx* gene(s) on the other chromosome(s).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a mutant plant source having a reduced range of starch grain morphologies (the starch grains for example being monodisperse or oligodisperse).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch is derived from a partial waxy mutant plant source.

Preferably, the activity of one or more GBSS isoenzyme(s) in the plant source is decreased or eliminated. The activity of two or more GBSS isoenzymes may be decreased or eliminated, and/or only one GBSS isoenzyme is active or expressed.

In particularly preferred embodiments, only one GBSS isoenzymes is active or expressed.

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the starch has been subjected to a physical (e.g., shear), biochemical (e.g., enzymic) and/or chemical (e.g., hydrolysis) treatment which is sufficient to render it non-retrograding, facilitates non-productive association between non starch polysaccharides (e.g., pentosans) and amylose such that amylose crystallisation (e.g., into the "B" form) is reduced or eliminated or is sufficient to reduce the chain length of the amylose to an extent sufficient to render it non-retrograding and/or to an extent sufficient to facilitate non-productive association between non starch polysaccharides (e.g., pentosans) and amylose such that amylose crystallisation (e.g., into the "B" form) is reduced or eliminated and/or to an extent to reduce or eliminate the ability of the amylose to crystallise after gelatinisation.

The starch of the invention may be derived from a hexaploid wheat variety in which the expression of the gene encoding GBSS on the A genome or the B genome or the D genome or the A and B genomes or the A and D genomes or the B and D genomes is reduced or eliminated.

Preferably, the expression of the gene encoding GBSS on the B genome is reduced or eliminated.

The starch is preferably derived from a hexaploid wheat variety in which the expression of the gene encoding GBSS on the D genome is increased relative to that of the gene(s) on the other chromosome(s).

In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the amylose is oligomorphous, each morphic form: (a) having a peak degree of polymerization selected from the

values set out above, and/or (b) being branched such that it has an average number of chains selected from the values set out above, and/or (c) having a molar fraction of branched amylose selected from the values set out above.

5 In another aspect, the invention provides a non-retrograding pourable food product comprising gelatinised starch, wherein the amylose is di- or oligomeric, the peak degree of polymerization and/or degree of branching and/or molar fraction of branched molecules being substantially homogeneous, similar or identical.

The food product is preferably a sauce, soup, food thickener or filling.

10 The plant source is preferably a cereal (e.g., wheat, rice, barley or corn), legume, tuber or root (e.g., potato).

In another aspect, the invention provides a process for producing a non-retrograding pourable food product comprising the steps of: (a) screening plant lines for a desired genotype, phenotype, starch or starch grain type having
15 characteristics as defined in any one of the preceding claims; (b) selecting a plant line having the desired characteristics identified in step (a); (c) formulating a pourable non-retrograding food product comprising starch extracted from plants bred from the plant line selected in step (b).

20 In another aspect, the invention provides a process for producing a high ratio cake flour comprising heating a flour, wherein the flour comprises starch which is ungelatinised but otherwise as defined above.

In another aspect, the invention provides a process for producing a high ratio cake flour comprising treating flour with chlorine gas, wherein the flour comprises starch which is ungelatinised but otherwise as defined above.

25 In another aspect, the invention contemplates the use of flour comprising starch which is ungelatinised but otherwise as defined above in the preparation of a high ratio cake flour mix.

The invention also provides a high ratio cake flour mix comprising starch which is ungelatinised but otherwise as defined above.

30 In another aspect, the invention contemplates the use of a composition (e.g., a flour) comprising gelatinised starch as defined above as a modified starch replacement.

In another aspect, the invention provides a non-staling food product (e.g., bread, bun, burger bun, sandwich) comprising starch as defined above.

35 In another aspect, the invention provides a method for producing a food product comprising the step of using starch as defined above as a modified starch replacer.

In another aspect, the invention provides a method of plant (for example cereal, e.g., wheat or legume, e.g., pea) breeding, comprising the step of screening

plants for a desired genotype, phenotype, starch or starch grain type having characteristics as defined above.

In another aspect, the invention provides a nucleotide or antibody (e.g., monoclonal antibody) probe for use in the breeding methods of the invention.

5 In another aspect, the invention provides a nucleotide probe (e.g., as defined above) for use in determining the *Wx* genotype of a plant (for example a cereal, e.g., wheat or a legume, e.g., pea).

In another aspect, the invention provides a nucleotide probe (e.g., as defined above) specific for the *Wx-A1*, *Wx-B1* or *Wx-D1*.

10 In another aspect, the invention provides an antibody (for example a monoclonal antibody) specific for *Wx-A1*, *Wx-B1* or *Wx-D1* proteins, for example for use in the breeding method of the invention.

In another aspect, the invention provides a nucleotide or antibody (e.g., monoclonal antibody) probe for use in determining the GBSS profile of a plant
15 (for example a cereal, e.g., wheat, or a legume, e.g., pea).

In another aspect, the invention provides a method of plant (for example cereal, e.g., wheat or legume, e.g., pea) breeding, comprising the step of screening plants by probing with an antibody or nucleotide probe of the invention.

In another aspect, the invention provides a freeze-thaw stable food product
20 comprising starch as defined above.

EXAMPLES

The invention will now be described in more detail with reference to several examples. These examples are for illustrative purposes only and are not intended to be limiting in any way.

25 EXAMPLE 1

A white wine and mushroom sauce was made to the following recipe:

Ike* wheat flour	8.00
Water	47.26
White wine	10.40
Sautéed chopped onions	10.00
Sliced mushrooms	8.00
Butter	5.54
Double cream	3.96
Sugar	1.50
Fat powder	1.50
Vegetable oil	1.50
White wine vinegar	1.00
Salt	0.90

Garlic granules	0.20
Lactic acid	0.15
Mushroom flavour	0.03
Dried parsley	0.03
White pepper	0.02
Butter acids	0.01

*IKE is a freely available hard red winter wheat variety produced by Kansas State University having null *Wx-A1* and *Wx-B1* alleles but a functional *Wx-D1* gene. It is described, for example, in Seeds and Crops Digest, the *Research News* section, January 1997.

5

All dry ingredients were dispersed in cold water, the wine, oil, vinegar and butter added and the mixture brought to the boil, with stirring, to gelatinise the starch. The sauce was then mixed intensively with a Silverson™ mixer for 1 min, the cream and lactic acid added, Silverson™ mixed for 4 min to achieve a fine dispersion of fat globules. The onion, mushroom and parsley were then added, and the sauce filled into glass jars. These were lidded and pasteurised by holding in a water bath at 90°C for 1 hour, and then cooled and stored at ambient temperature.

After one month's storage, the sauce was smooth and pourable. When used a medium in which to cook chicken pieces at 1 hour in an oven at 175°C, the product was smooth and of appropriate viscosity.

COMPARATIVE EXAMPLE 1

A white wine and mushroom sauce was made according to the recipe described in Example 1, except that the Ike flour was replaced with 2.00% modified starch (Colflo 67™) and the water adjusted accordingly. After storage, the sauce was smooth and pourable.

COMPARATIVE EXAMPLE 2

A white wine and mushroom sauce was made according to the recipe described in Example 1, except that the Ike flour was replaced with 6.00% standard wheat flour. After storage, the sauce formed a solid gel and was difficult to remove from the jar.

EXAMPLE 2

A mushroom soup was made to the following recipe:

Ike flour	6.00
Water	82.45
Sliced mushrooms	10.00
Double cream	2.50

Mushroom concentrate	2.25
Cream powder	1.00
Sodium caseinate	1.00
Flavour	0.05
Ground white pepper	0.02

- 5 The dry ingredients were first mixed with the cold water, and the mushroom concentrate then mixed in. The mixture was heated to 85°C, with stirring, to gelatinise the starch. The cream and mushrooms were then added. The product was then filled into cans and sterilised by retorting at 120°C for 1 hour, cooled in water and stored at ambient temperature.

After one week's storage, the cans were opened and the soup examined. It poured easily and smoothly and had a good, clean flavour typical of freshly made soup on reheating and tasting.

10 COMPARATIVE EXAMPLE 3

A soup was prepared according to the recipe described in Example 2, except that the Ike flour was replaced with 6.00% standard wheat flour. After storage, the soup formed a solid gel and was difficult to remove from the can.

COMPARATIVE EXAMPLE 4

- 15 A soup was prepared according to the recipe described in Example 2, except that the Ike flour was replaced with 2.00% modified starch and the water adjusted accordingly. After storage, the soup poured easily and smoothly. However, it had an unpleasant off-flavour after heating and tasting.

EXAMPLE 3

- 20 A tomato sauce for pasta was made according to the following recipe:

Ike flour:	3.25
Water:	37.51
Canned tomatoes:	25.00
Tomato puree:	15.00
Chopped onion:	6.00
Pureed onion:	4.00
White wine:	3.00
Sugar:	3.00
Salt:	1.20
Vegetable oil:	1.00
Garlic granules:	0.50
Olive oil:	0.25
Parsley:	0.06

Thyme:	0.06
Oregano:	0.04
Black pepper:	0.02

All dry ingredients were mixed in cold water and the wine, oil and tomato puree then mixed in. The sauce was heated to 85°C to gelatinise the starch, and the tomatoes, herbs and onions added. The temperature was brought back up to 85°C and the sauces filled into glass jars and pasteurised in a water bath at 90°C for 1 hour. The samples were cooled and stored at ambient temperature.

When assessed after one month, the sauce had a pulpy, pourable consistency, was a natural red colour and showed no sign of syneresis. On heating and tasting, it had a clean, non-starchy flavour.

10 COMPARATIVE EXAMPLE 6

A sauce was prepared according to the recipe described in Example 3, except that the Ike flour was replaced with 2.00% modified starch and the water adjusted accordingly. After storage, the sauce poured easily and smoothly. However, it had a deep red colour and a starchy, less clean flavour than the sauce prepared in Example 3.

15 COMPARATIVE EXAMPLE 7

A sauce was prepared according to the recipe described in Example 3, except that the Ike flour was replaced with 3.25% standard wheat flour. After storage, the sauce was pourable but showed clear signs of syneresis. It also had a more starchy, less clean flavour than the sauce prepared in Example 3.

EXAMPLE 4

Ike wheat flour was milled for use as a cake flour. A portion of the flour was dry heat treated to increase the swelling capacity of the starch. High ratio cakes were then made according to the following recipe:

25

Untreated Ike flour:	100
Sugar:	120
High ratio shortening (Dexo™):	75
Whole egg:	80
Milk:	60

The fat and sugar were creamed together in a Hobart™ mixer, the eggs added with beating, and then the flour and milk gently mixed in. The batter was scaled and 400 g was placed into eight inch round cake tins. The cakes were baked at 180°C for 25 minutes. After baking, the cakes kept their shape and did not collapse.

30

EXAMPLE 5

Cakes were baked according to the procedure described in Example 4, except that all of the Ike flour was heat treated. The volume and internal structure was improved compared to the cakes prepared in Example 4.

5

COMPARATIVE EXAMPLE 8

Cakes were made according to the procedure described above for Examples 4 and 5, except that standard cake flour was used in place of the Ike flours. The cakes collapsed and were unacceptable.

COMPARATIVE EXAMPLE 9

10

Cakes were made according to the procedure described above for Examples 4 and 5, except that chlorinated high ratio cake flour was used in place of the Ike flours. The cakes were comparable in all respects to those produced in Example 5.

EXAMPLE 6

15

Aqueous flour dispersions of mass 5 kg were prepared in a Hobal steam jacketed vessel. The flour addition rate was chosen such as to give a starch solids content of 5% in the mix. The product was stirred gently during the heating process via scraped surface paddles. The sample temperature was raised to 95°C and held for 5 minutes to gelatinize the starch. A sample of mass 1.5 kg was then transferred to a "Tweedy" type mixing bowl attached to the Compudomixer. The "Tweedy" bowl (usually used for dough mixing) comprises a cylindrical chamber. Mixing is achieved via a spiral rotor, driven from below, via a gearbox attached to the Compudomixer. The sample is sheared and thrown against three baffle plate fixtures attached to the wall of the mixing vessel. The Compudomixer was used to control the quantity and rate of energy input into the sample.

20

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The hot product was mixed at a fixed rate of work input 2 kJ/kg/min to work input levels encompassing the range from 0-60 kJ/kg. The mixing bowl temperature was maintained at 80°C via a recirculating water bath throughout the mixing study. Subsamples of mass 60 g were removed at intervals for rheological testing and retrogradation studies (stored in plastic pots) for 23 hours at a temperature of 5°C. The rate of work input settings and work levels were reprogrammed following subsampling to maintain a constant rate of work input to the sample during mixing.

30

35

The products were removed following 23 hours refrigerated storage at a temperature of 5°C. The temperature was chosen to maximize the rate of amylose retrogradation/gelation. The amylopectin retrogradation is characterised as occurring at a lower rate over an extended time period. The pots were allowed one hour to equilibrate to ambient temperature (20°C), prior to subjective visual

assessment and objective rheological studies using the Rheometrics Fluids Spectrometer (RFS).

The results of a subjective comparison between a standard wheat flour and IKE flour is summarized in the following table:

5

**TABLE 1: PROPERTIES OF "RETROGRADED" FLOUR PASTES
FOLLOWING 23 HOURS STORAGE AT 5°C**

Work input (kJ/kg)	Standard flour	IKE flour
0	Firm set gel, breaks into chunks on cutting	Thick paste, disperses with minimal shear
10	Set gel	Smooth, pourable syrup
20	Weaker gel	Pourable, low viscosity syrup
30	Very weak, breaks down into paste on cutting	Pourable, low viscosity syrup
40	Breaks down on spooning from jar	Pourable, low viscosity syrup
50	Pourable syrup	Very low viscosity syrup
60	Smooth pourable syrup	Very low viscosity syrup

The standard wheatflour product in the absence of shear post gelation gave a firm set gel on refrigerated storage overnight. The gel strength was found to decrease with increasing work input. However, at best, the product required something of the order of 40 kJ/kg work input to give a product which would pour from the jar, after gentle shaking. In contrast, the unsheared partial waxy IKE-based product did not give the firm set gel which is characteristic of standard gelled wheatflours. The product, although appearing to have set, could be described as having the consistency of a very viscous paste. It did not fracture on cutting, giving "clean cut" edges as was the case for the standard wheatflour equivalent. A pourable product was obtained at the lowest work input setting of 10 kJ/min, and pourable sauce products were also obtained at much lower work inputs.

The RFS was used to measure the rheological properties of the products following storage at a temperature of 5°C. The effect of energy input during mixing on the viscosity of the stored products is illustrated in Figure 3. The "unsheared" products, although visually appearing quite different, yielded similar viscosity values. It is clear from the data that the partial waxy product was more sensitive to the energy input during mixing than the "standard" wheatflour.

CLAIMS:

1. A non-retrograding food product which is pourable, liquid or spoonable, the food product comprising gelatinised starch, wherein the starch is derived from a plant source in which the amylose content of the starch granule is reduced (but not entirely eliminated).
2. The food product of Claim 1 which:
 - (a) is non-stringy; and/or
 - (b) is non-mucilaginous; and/or
 - (c) has a low degree of elasticity.
3. The food product of Claim 1 or Claim 2 wherein the plant source exhibits partially depressed amylose synthase activity.
4. The food product of any one of Claims 1 to 3 wherein the starch is a partial waxy mutant starch.
5. The food product of Claim 4 wherein the mutant starch is derived from a plant which has been genetically or environmentally manipulated.
6. The food product of Claim 5 wherein the plant has been genetically manipulated to decrease or eliminate the activity of one or more GBSS isoenzymes.
7. The food product of Claim 5 wherein the plant has been environmentally manipulated by regulation of water availability, temperature, fertilization or light.
8. The food product of any one of the preceding claims wherein the starch is derived from a plant source which is selected from a cereal (e.g., wheat, rice, barley or corn), legume (e.g., pea), tuber or root (e.g., potato).
9. The food product of Claim 8 wherein the plant source is a wheat variety in which the expression of the gene encoding GBSS on:
 - (a) the A genome; or
 - (b) the B genome; or
 - (c) the D genome; or
 - (d) the A and B genomes; or
 - (e) the A and D genomes; or
 - (f) the B and D genomes,is reduced or eliminated.
10. A food product as defined in any one of the preceding claims wherein the food product is:
 - (a) a babyfood; or
 - (b) a bakery supply product (for example, a custard or a bakery filling or topping); or
 - (c) a fruit filling; or



- (d) a gravy, soup, sauce or food thickener;
(e) a frozen, chilled or ambient stable gravy, soup, sauce or food thickener; or
(f) a pasteurised, retorted or UHT treated gravy, soup, sauce or food thickener; or
(g) a meal or meal component; or
(h) a petfood; or
(i) a dairy product (e.g., a dessert or yogurt); or
(j) a salad dressing; or
(k) a spread.

11. Use of a composition (e.g., a flour) comprising ungelatinised starch as defined in any one of Claims 1-9 as a modified starch replacement, or a modified starch replacer comprising ungelatinized starch as defined in any one of Claims 1-9.

12. A non-staling food product (e.g., bread, bun, burger bun, sandwich) comprising starch as defined in any one of Claims 1-9.

13. A method for producing a food product comprising the step of using starch as defined in any one of Claims 1-9 as a modified starch replacer.

14. A method of thickening a food product comprising the step of:
(a) adding a composition (e.g., flour) comprising ungelatinised starch which is otherwise as defined in any one of Claims 1-9;
(b) gelatinising the starch to thicken the food product.

15. A thickened food product obtainable by (or produced by) the method of Claim 14.

16. A method for retarding staling of a food product (e.g., a baked food product, such as bread, e.g., a burger bun or sandwich) comprising the step of formulating the food product with starch as defined in any one of Claims 1-9.

17. A method for retarding retrogradation of a food product comprising the step of formulating the food product with starch as defined in any one of Claims 1-9.

18. A dehydrated food base comprising ungelatinised starch which is otherwise as defined in any one of Claims 1-9.

19. An instantisable food base (for example, a roux e.g., a roux blanc) comprising ungelatinised starch which is otherwise as defined in any one of Claims 1-9.

