Title: MILK PRODUCT AND METHOD FOR ITS PREPARATION

Abstract: A method of using tyrosinase for modifying the structure of milk products prepared from fat-reduced milk is disclosed. The method may be applied to raw or pasteurized fat-reduced milk, and it is used especially for preparing acidified milk products such as e.g. yogurt. Milk products with tyrosinase-modified structure are also disclosed.
Milk product and method for its preparation

Field of the Invention

The present invention relates to milk products with improved structure. More specifically it relates to a method of using a particular enzyme for modifying the structure of milk products. The invention especially relates to preparing acidified milk products such as yogurt. The invention enables the use of raw or pasteurized milk instead of more severely heat-treated milk.

Background of the Invention

Milk products constitute an essential nutritional source in the human diet. Milk is an excellent protein source, and in addition it usually contains minerals, such as calcium, and vitamins. Acidified milk products that are conventionally prepared by lactic acid bacterial fermentation may further comprise health-promoting lactic acid bacteria. Milk products also contain animal fats, which have an important contribution to the mouthfeel of the products.

In addition to the chemical and biological ingredients of a milk product, also the physical properties such as viscosity, thickness, firmness and water-holding capacity have a great influence on the quality, and especially the texture of the product. Texture is not only related to sensory perception but also to water holding capacity, gelling, emulsifying properties and stability.

Separation of water from a product is a phenomenon called syneresis, which may be associated with the preparation of acidified milk products. Syneresis is undesirable and should be avoided or at least minimized. A number of ways for improving the physical properties of food products have been described.

Milk gels are formed during acidification due to casein gelation. Heat treatment before acidification has been considered an important step for the texture of the acid milk product (Pereira et al., 2003). If milk is not heated only casein contributes to gel formation. After heat-induced denaturation whey proteins take part in the texture formation of acid milk gels both by associating partly with casein micelles, and by forming whey protein aggregates dispersed in the continuous casein network (van Vliet et al., 2004).

Another approach to improve the structure of yogurt and other milk products is to elevate the protein content, or add thickening agents such polysaccharides, pectin, gelatin and other hydrocolloids, and starch. The thickening agents increase the viscosity of the yogurt, but unfortunately the palatability
may decrease due to a g!uey mouthfeel. A further disadvantage is the customers' negative attitude towards food additives in general.

A more natural way of improving the properties of milk products is to modify them enzymaticalSy. Enzymes can be used for modification of the technologicaS properties of foodstuffs e.g. by creating covalent cross-links between amino acid residues in proteins or by enzymes oxidizing certain amino acid residues. Oxidation of amino acid residues can in turn also result in formation of cross-üinks. Modification of proteinaceous material by cross-linking is frequently used in food processing.

Enzymes suggested for food applications include transglutaminase, lipoxygenase, polyphenol oxidase (tyrosinase), peroxidase, lysyl oxidase, protein disulfide isomerase and sulfhydryl oxidase (Matheis and Whitaker, 1987). Further proposed enzymes are laccase, bilirubin oxidase, ascorbic acid oxidase and ceruloplasmin, which have been proposed for cross-linking e.g. plant and animal proteins such as cereal proteins, casein, β-lactoglobulin, and egg proteins (US2002/000970). The applicability of a particular cross-linking enzyme for a particular use depends on a number of factors, such as availability and accessibility of substrate, possible interfering compounds, addition of external substrates, pH, temperature, inhibitors etc.

Currently, transglutaminases (TG, glutamylpeptide:amine γ-glutamyltransferase, EC 2.3.2.13) are the only intensively studied and commercially available enzymes for cross-linking of milk proteins. The reactivity of transglutaminase is dependent on the availability and accessibility of the target amino acids glutamine and lysine in the protein substrate.

The main proteins of cow milk consist of a₅, a₂ς, β- and κ-caseins, and the globular whey proteins a-lactaSbumin (a-la), β-lactoglobulin, (β-Lg). Casein is considered a very good substrate for transglutaminase, whereas globular whey proteins, i.e. α-La and β-Lg, on the other hand have been shown to be poor substrates. Partial unfolding of globular proteins by chemical modification, with reducing agents or by thermal treatment has improved their susceptibility to cross-linking by transglutaminase.

Nonaka et al., 1992 have studied the reactivity of transglutaminase to casein in skim milk powder, and compared it to its reactivity to caseinate. They found that the reactivity of transglutaminase to casein in the milk was inferior to that to caseinate, which indicates that casein in milk is not susceptible to enzymatic cross-linking in the same way as caseinate.
It has been shown that heating the milk prior to cross-linking with transglutaminase dramatically increases the reactivity of the milk proteins. Preheating at 85°C for 15 minutes has been found to enhance the susceptibility of milk proteins to cross-linking (Sharma et al. 2001). It has further been found that cow milk heat treated at low temperature (63°C, 30 min.) had a lower susceptibility to transglutaminase than cow milk sterilized at 130°C (2-3 sec). When the former was additionally heat-treated to 90°C, the reactivity to transglutaminase significantly improved. Preheating raw milk to 95°C for 2 seconds before reaction with transglutaminase has also been reported to be effective in yoghurt production (US2002/0061358).

However, too severe heat-treatment before acidification and/or enzymatic treatment is also associated with disadvantages. First the heat-treatment is an additional step that complicates the production of the milk product, and of course the time and energy needed increases the production costs. Further the heat-treatment denatures the milk proteins, which leads to a loss of flavor, bitter taste, and an adverse effect on the mouthfeel.

It has been reported that heat-treatment may be avoided in connection with transglutaminase by adding a reducing agent such as a thiol compound to the milk. The reducing agent enables the use of transglutaminase on raw milk without the need of preheating. Suitable reducing agents are e.g. glutathione, cysteine, γ-glutamylcysteine, sulfurous acid, ascorbic acid and erythorbic acid. Milk treated with transglutaminase in the presence of a reducing agent has been suggested for use in the production of yogurt, cheese and powdered milk to improve texture and mouthfeel (US2002/0061358). However, the addition of a reducing agent may also be problematic, because food additives should be avoided whenever possible. Further, the reducing agent may influence other properties of the final product, such as taste or flavor.

The present invention relates to a new method of producing structure modified milk products without the need of detrimental preheating, or adding reducing agents, which are associated with transglutaminase.

Tyrosinase is another cross-linking enzyme that has been suggested for food applications. Tyrosinase has been reported to affect for example wheat proteins (Takasaki and Kawakishi, 1997; Takasaki et al., 2001). Lantto et al. (in press) have studied the effect of transglutaminase, tyrosinase and freeze-dried apple pomace powder on gel forming and structure of homogenized pork meat. Tyrosinase was not able to affect gel forming in the ex-
periments conducted, but it improved gel hardness of an unhealed meat homogenate to a certain extent.

Tyrosinase has further been reported to cross-link the whey proteins a-La and β-Lg in the presence of caffeic acid. In contrast to β-Lg a-La was even capable of direct cross-linking by tyrosinase without addition of caffeic acid (Thaimann and Loetzbeiner, 2002). Further the effect of tyrosinase on casein in the presence or absence of L-tyrosine and L-DOPA as cross-linkers has been studied. The presence of cross-linkers was found to be indispensable for the cross-linking of casein (HalaouSi et al., 2005).

None of the above references disclose the use of tyrosinase in cross-linking the proteins in milk. Applicants tried to do this, but with poor result. As could be seen with transglutaminase isolated milk proteins do not react in the same way as they do in milk. Tyrosinase did not show significant cross-linking activity in whole cow milk. Preheating the milk did not significantly enhance cross-linking. Surprisingly, however, it was found that reduction of the fat content of the milk resulted in desired modification of the texture of the product when using tyrosinase. This was especially surprising, because fat reduction generally has an adverse effect on the texture and water holding of the product.

The present invention is directed to a method of modifying milk proteins, which is suitable for treating raw, or moderately heat-treated, fat-reduced milk, and which has superior texture modifying and syneresis preventing effects compared to transglutaminase. The method is convenient for preparing acidified milk products, and it enables the use of pasteurized milk instead of using pasteurized and conventionally reheated milk, as well as the preparation of milk products with reduced fat and protein content without the need of other additives, such as thickening agents.

In processed milk products fat and added proteins, starches and polysaccharides greatly affect the technological and sensory properties. The present invention now contributes to the preparation of healthier, less additives containing low-energy milk products suitable for e.g. weight management.

Summary of the Invention

The present invention provides a method of preparing a milk product, said method comprising adding tyrosinase to raw or pasteurized faK reduced milk and incubating it to form a milk product with modified structure.
The invention further provides the use of tyrosinase in modifying the structure of raw or pasteurized fat-reduced milk.

The invention still further provides a milk product comprising raw or pasteurized fat-reduced milk and tyrosinase, and having a tyrosinase modified structure.

Specific embodiments of the invention are set forth in the dependent claims.

Other objects, details and advantages of the present invention will become apparent from the following drawings, detailed description and examples.

Brief Description of the Drawings

Figure 1 shows an SDS-PAGE gel of non-heated and preheated (90°C) skim milk without tyrosinase treatment, or with 500 nkat tyrosinase/g of protein.

Figure 2 shows the effect of tyrosinase on the firmness of a 2.7% Na-caseinate gel.

Figure 3 shows the effect of tyrosinase (TYR) on the firmness of an acidified milk gel prepared from pasteurized whole milk or skim milk.

Figure 4 shows the effect of tyrosinase (TYR) on the firmness of an acidified milk gel prepared from pasteurized skim milk.

Figure 5a shows the effect of tyrosinase (TYR) and transglutaminase (TG) on the firmness of an acidified milk gel prepared from pasteurized skim milk.

Figure 5b shows the effect of tyrosinase (TYR) and transglutaminase (TG) on liquid released from an acidified milk gel prepared from pasteurized skim milk.

Figure 6 shows an amino acid sequence alignment of two *Trichoderma reesei* tyrosinases TYRI and TYRIL. The sequences are shown up to the C-terminal cleavage site of TYRII. The signal sequences are on the first row. A putative propeptide cleavage site of TYRI is shown by an arrow. The amino acid residues involved in coordination of the two Cu atoms in the active site are shaded. The thioether bond between cysteine and histidine involved in the active site of the tyrosinases is shown by a vertical line above the sequence.
Detailed Description of the Invention

Tyrosinase belongs to the group phenol oxidases, which use oxygen as electron acceptor. Traditionally tyrosinases can be distinguished from other phenol oxidases i.e. laccases on the basis of substrate specificity and sensitivity to inhibitors. However, the differentiation is nowadays based on structural features. Structurally the major difference between tyrosinases and laccases is that tyrosinase has a binuclear copper site with two type III coppers in its active site, while laccase has altogether four copper atoms (type I and II coppers, and a pair of type III coppers) in the active site.

Tyrosinase oxidizes various phenolic compounds to the corresponding quinones. The quinones are highly reactive and may react further non-enzymatically. A typical substrate of tyrosinase is tyrosine (or tyrosine residue in proteins), which is first hydroxylated into DOPA (dihydroxyphenylalanine or DOPA residue in proteins), which is then further oxidized by the enzyme to dopaquinone (or dopaquinone residue in proteins). Dopaquinone may react non-enzymatically with a number of chemical structures such as other dopaquinones, thiol and amino groups. Tyrosinase thus has two enzyme activities in one and the same protein i.e. monophenol monooxygenase activity (EC 1.14.18.1) and catechol oxidase activity (EC 1.10.3.1) as shown below.

The substrate specificity of tyrosinase is relatively broad, and the enzyme is capable of oxidizing a number of polyphenols and aromatic amines. Contrary to laccase (EC 1.10.3.2), however, tyrosinase does not oxidize syringaldazin. At least tyrosine, lysine and cysteine residues in proteins form covalent bonds with dopaquinones formed by tyrosinase.

Tyrosinase activity can be measured by techniques generally known in the art. L-DOPA or L-tyrosine can be used as a substrate, whereafter dopachrome formation may be monitored spectrofotometrically, or alternatively
substrate consumption may be monitored by following the oxygen consump-
tion.

Tyrosinases are widely distributed in nature, and they are found in
animals, plants, fungi (including mushrooms) and bacteria. The only com-
cially available tyrosinase at present is derived from the mushroom Agaricus
bisporus. The tyrosinase used in the present invention may originate from any
animal, plant, fungus or bacteria capable of producing tyrosinase. According to
one embodiment of the invention the tyrosinase is of microbial origin. Tyrosi-
nases may be found e.g. in Neurospora crassa, Streptomyces, Bacillus, My-
rotheium, Mucor, Mihococcum, Aspergillus, Chaetomastia, Ascovaginospora,
Chaetomium, Trametes, Serpula lacrymans, Conidiophora puteana,
Pycnoporus, Scytalium and Th choderma. According to a specific embodiment
of the invention the tyrosinase is derived from a filamentous fungus. Suitable
tyrosinases are described in PCT/FL2006/050055. The tyrosinase may for ex-
ample be an extracellular tyrosinase obtainable from Th choderma reesei, or a
tyrosinase variant thereof. According to one particular embodiment of the in-
vvention the tyrosinase comprises an amino acid sequence having at least 70,
80, 90, 95, 98 or 99% identity to the amino acid sequence of TYR1 or TYR2 as
shown in Figure 6, or to a fragment thereof having tyrosinase activity.

An effective amount of tyrosinase is added to raw or pasteurized fat-
reduced milk under conditions sufficient to modify the structure of the milk
product obtained. The structure of a milk product refers both to its chemical
and physical properties. Structure influences the sensory perception of the
product i.e. the texture. The relationship between structure and water-holding
capacity is complicated and not straightforward, but "structure" as used herein
includes both texture and water-holding properties.

The milk to be treated with tyrosinase is obtained from milking ani-
mals, preferably from cows or goats. Before enzyme treatment fats and oils are
removed to obtain fat-reduced milk. Milk may contain up to about 10% fat, but
normally whole milk has a fat content of 3.5-3.6 weight-%. "Fat-reduced milk"
in the present context refers to milk having a maximum fat content of 2.0% by
weight. Preferably it has a fat content of no more than 1.5, 1.0, or 0.5 weight-
%. According to a specific embodiment of the invention all fat is removed to ob-
tain "skim milk" having a fat content of 0% by weight. The preparation of fat-
reduced including skim milk is general knowledge.
"Raw" milk as used herein refers to milk that has not been heat-treated to decrease its biological burden.

"Pasteurized" milk refers to milk that has been moderately heat-treated to destroy pathogens, undesirable enzymes and milk spoilage bacteria. Pasteurization is a generally used term in the dairy industry, and refers to a minimum of heat treatment needed for obtaining a microbiologically safe product with prolonged shelf life. The extent of microorganism and enzyme inactivation depends on the combination of temperature and time. Heat-treatment at 62 to 65°C for about 30 minutes is used in some countries, whereas other use temperatures between 70 to 75°C for much shorter times. Milk is normally pasteurized at 72°C for 15 seconds. Milk is deemed pasteurized if it tests negative for phosphatase but positive for peroxidase.

Both the raw and the pasteurized milk may have undergone other processing, such as centrifugation, filtration or homogenization before pasteurization and/or the treatment with tyrosinase. Conventional food additives may be added, if desired.

The tyrosinase modified fat-reduced milk may be used e.g. in preparing milk powder by spray drying. According to a particular embodiment of the invention the tyrosinase modified fat-reduced milk is used in the preparation of acidified milk products, such as yogurt, clabber milk, desserts, crème fraiche, ripened cream and the like. The preparation of these products is carried out without rennet or other proteolytic enzymes. The "milk product" formed by the method of the invention may thus be e.g. a milk concentrate or powder, yogurt, clabber milk, crème fraiche, ripened cream, fat-reduced cream, a dairy dessert, or the like.

As set forth above, heat treatment before acidification is considered essential for the texture of acid milk products. If transglutaminase is used, the heat treatment is further necessary for enhancing the susceptibility of milk proteins to cross-linking. "Heat treatment" or "preheating" in this context is harsher than pasteurization and leads to peroxidase inactivation. The conventional preheating temperatures for different processes may vary between 85 and 140°C, and the treatment times may vary from 30 minutes down to a few seconds, usually 85-95°C for 30-10 minutes is used. Skim milk is normally preheated at about 85 to 95°C for about 5 to 15 minutes, the higher the temperature the shorter the time.
Milk contains naturally about 3.2% protein. Yogurt is conventionally prepared from milk having a protein content of at least 3-5% by weight depending on the amount of other additives used, such as hydrocolloids and starch to obtain the desired texture. If additives are not used, the protein content is elevated up to about 5% e.g. by evaporation or by adding external protein. Pasteurized milk with optionally elevated protein content is heat-treated at about 90-95 °C for about 5-10 minutes, and sugar, fruit, jam or other tasters may be added. The mixture is then cooled and inoculated with a starter i.e. acid producing microorganisms such as lactic acid bacteria, which contribute both to acidification and taste. Fermentation is normally carried out at 40-45 °C for 3-6 hours. Possible enzymes are added prior to or concomitantly with the starter. After fermentation the yogurt may optionally be heat-treated once more to prolong its storage time. However, this postheating may also be avoided in order not to destroy the fermentation organisms of the final product, which might have health-promoting properties. For experimental acidification D-gluconic acid lactone (GDL) may be used instead of fermenting organisms.

According to the present invention acidified milk products may be prepared with tyrosinase in the same way as described above, except that no heat treatment exceeding 80 °C is carried out before acidification. If the milk has been pasteurized already at the farm, it may optionally be repasteurized in the dairy at a temperature not exceeding 80 °C before the enzyme treatment. The tyrosinase may be added before or after the addition of the starter. Conveniently the tyrosinase is added simultaneously with the starter and allowed to react during the lactic acid fermentation. The reaction mixture may be further supplied with oxygen to enhance the effect of the enzyme. Tyrosinase enables the use of milk with a protein content of less than 5 weight-%, and especially less than 4 weight-% e.g. with a natural protein content of about 3.2% for yogurt preparation.

Tyrosinase is dissolved in an aqueous solution. An amount of at least 10, 20, 40, 80, 120, 160, 320, or 640 nkat/g milk protein is usually sufficient to modify the texture and/or water-binding properties of the milk product. Tyrosinase is normally allowed to react at a temperature of about 4-40 °C for at least 10 minutes up to 24 hours or more. Naturally incubation at low temperatures requires longer incubation times and vice versa. An incubation time of at least 1 hour up to at least 18 h is convenient at 4 °C, whereas reaction times of at least 10 minutes up to 4 hours at 40 °C are efficient. The pH may range from
3 to 8, preferably from 3 to 5 during the process. If desired, the incubation may be terminated with postheating, but this is not necessary.

Tyrosinase especially modifies the texture and the water-holding capacity of the milk product. The effect of tyrosinase on the proteins in fat-reduced milk can be seen e.g. as increased molecular weight of milk proteins, increased firmness measured as maximum compression force or area, and/or improved water holding capacity. Water holding capacity may be measured e.g. as liquid released from the cross-linked gel.

According to a particular embodiment of the invention raw or pasteurized skim milk is inoculated with microorganisms capable of forming lactic acid to obtain a fermentation mixture, and the mixture is incubated in the presence of tyrosinase under conditions sufficient to acidify the milk product and modify the texture and water-holding capacity thereof.

The invention is illustrated by the following non-limiting examples.

Example 1. Tyrosinase catalysed cross-linking of heated and non-heated milk products

A tyrosinase gene encoding a protein comprising the sequence of TYR2 as shown in Figure 6 was amplified by PCR from genomic DNA of *Trichoderma reesei*, and the isolated gene was overexpressed in *T. reesei* under a CBHI promotor, and excreted into the growth medium.

Tyrosinase activity was assayed using 15 mM L-DOPA (Sigma, USA) as substrate at pH 7 and room temperature according to Robb (1984). Enzyme activity is expressed in nanokatals (nkat). One nkat is defined as the amount of enzyme activity that converts one nmol per second of substrate used in the assay conditions. Enzyme dosage nkat/g protein means the amount enzyme calculated as activity and dosed per one gram of milk protein.

Changes in the molecular weight and mobility of milk proteins caused by the *T. reesei* tyrosinase were analysed by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Tyrosinase was added to raw or heated (90°C, 5 min) skim milk at a dosage of 500 nkat/g protein in open tubes (oxygen available) and closed tubes (less oxygen available). Incubation was carried out at 30°C for 2 hours.
The results are shown in Figure 1. The samples were as follows: lanes 1 and 10: molecular weight standard, lane 2: unheated milk in closed tube, lane 3: unheated milk + tyrosinase in closed tube, lane 4: unheated milk in open tube, lane 5: unheated milk + tyrosinase in open tube, lane 6: heated milk in closed tube, lane 7: heated milk + tyrosinase in closed tube, lane 8: heated milk in open tube, lane 9: heated milk + tyrosinase in open tube, lane 10: molecular weight standard.

Tyrosinase caused the following detectable electrophoretic changes: 1) Appearance of high molecular weight compounds around 198 KDa and below the well, 2) disappearance of caseins (especially in open tubes). The results show that tyrosinase was capable of catalyzing cross-links formation of both unheated and heated milk, the effect being higher in the presence of oxygen.

Example 2. Improvement of firmness of Na-caseinate protein gels by tyrosinase

Gels were prepared from Na-caseinate dissolved in tap water over night at room temperature. Caseinate solutions were chemically acidified with GDL (D-gluconic acid lactone; 0.4% GDL in 2.7% (w/w) caseinate, final pH 4.6-4.7) during the enzyme treatment. *T. reesei* tyrosinase (0, 10 and 50 nkat/g caseinate) and GDL were mixed to 13 ml of caseinate solution and 2.35 ml aliquots of the solution were pipetted in cylindrical aluminium vessels (cross section 16 mm). The samples were covered with a glass Petri dish and they were incubated at 30°C for 4 hours. Firmness of the 1 day old gels was measured at room temperature (Texture Analyzer TA-HDi, Stable Micro Systems) by measuring the maximum compression force needed to compress the gels 5 mm (0.5 mm/s, 4 replicate samples). For comparison the firmness of a 4.5% caseinate gel without enzyme treatment was measured. The results are shown in Figure 2.

Tyrosinase increased the gel firmness at both dosages. Further the firmness of the tyrosinase-treated 2.7% caseinate gel was almost the same as that of a 4.5% caseinate gel without tyrosinase; max compression force for both samples was around 100 g, indicating that the protein content of the gel can be decreased by using tyrosinase.
Example 3. Tyrosinase catalyzed cross-linking of whole milk and skim milk

Tyrosinase from *T. reesei* was added to pasteurized whole milk (3.5% fat) and skim milk (0% fat) at a dosage of 50 nkat tyrosinase/g protein at a milk temperature of 40°C, and the milk was incubated at 40°C for 1 h. During incubation, the samples were continuously supplied with oxygen. Also a control solution without enzyme was prepared. Then the milk sample was acidified with 1.2% GDL at 40°C for 4 h (final pH 4.6). After over night storage at 4°C, the maximum compression force was measured with a Texture Analyzer (TA-HDi, Stable Micro Systems). The results are shown in Figure 3. Tyrosinase greatly increased the firmness of the acidified milk gel. The effect was significantly greater with skim milk than with whole milk.

Example 4. Tyrosinase-induced cross-linking of pasteurized skim milk

Mushroom tyrosinase (Fluka, Switzerland) was added (50 nkat tyrosinase/g protein) to warm (40°C) pasteurized skim milk and the milk was incubated at 40°C for 1 h. During incubation, the sample was continuously supplied with oxygen. Also a control solution without enzyme was prepared. Then the milk sample was acidified with 1.2% GDL at 40°C for 4 h (final pH 4.6). After over night storage at 4°C, the maximum compression force was measured with a Texture Analyzer (TA-HDi, Stable Micro Systems). The results are shown in Figure 4. Mushroom tyrosinase increased the hardness of the acidified skim milk gel.

Example 5. Comparison of tyrosinase with transglutaminase

Tyrosinase from *T. reesei*, or transglutaminase (Activa MP, Ajinomoto) was added to warm (40°C) pasteurized skim milk both at a dosage of 50 nkat enzyme/g protein and the milk was incubated at 40°C for 1 h. Tyrosinase activity was determined as described in Example 1, and transglutaminase activity was determined using 0.2 M N-carbobenzoxy (CBZ)-L-glutaminylglysine (Sigma, USA) as the substrate at pH 6 (Folk, 1970). One nkat is defined as the amount of enzyme activity that converts one nmol per second of substrate used in the assay conditions. Enzyme dosage nkat/g protein means the amount enzyme calculated as activity and dosed per one gram of milk protein.

During incubation, the sample containing tyrosinase was continuously supplied with oxygen. Also a control solution without enzyme was prepared. Then the milk sample was acidified with 1.2% GDL at 40°C for 4 h (final
pH 4.6). After overnight storage at 4°C, the liquid released from the gel was gently poured out from the sample cup and weighed. After that, the maximum compression force was measured with a Texture Analyzer (TA-HDi 9 Stable Micro Systems).

Although the enzyme activities of tyrosinase and transglutaminase are not directly comparable due to differences in reaction mechanisms and model substrates, it could be seen that tyrosinase was superior to transglutaminase.

The results of the compression test are shown in Figure 5a. The firmness of the acid milk gel was greatly increased by the use of tyrosinase, while the gel containing transglutaminase was only slightly firmer than the control gel without enzyme.

The amount of liquid released from the gels during overnight storage at 4°C is shown in Figure 5b. The water binding of the acid milk gel was reduced to zero by the use of tyrosinase. Transglutaminase did not prevent the liquid released compared to the no enzyme control.
References


Claims

1. Method of preparing a milk product, said method comprising adding tyrosinase to raw or pasteurized fat-reduced milk and incubating it to form a milk product with modified structure.

2. The method of claim 1, wherein an acidified milk product is prepared.

3. The method of claim 2, wherein the acidified milk product is yogurt, clabber milk, crème fraîche, ripened cream or a dessert.

4. The method of claim 1, wherein milk concentrate, milk powder or fat-reduced cream is prepared.

5. The method of claim 1, comprising inoculating raw or pasteurized skim milk with microorganisms capable of forming lactic acid to obtain a fermentation mixture, and incubating said mixture in the presence of tyrosinase under conditions sufficient to acidify the milk product and modify the texture and water-holding capacity thereof.

6. The method of claim 1, wherein the tyrosinase is of fungal origin.

7. The method of claim 1 for preparing an acidified milk product, preferably yogurt having a fat content of no more than 2%, and a protein content of less than 4%.

8. The method of claim 1, wherein the fat-reduced milk has been heat-treated at a temperature not exceeding 80°C before the addition of tyrosinase.

9. Use of tyrosinase in modifying the structure of raw or pasteurized fat-reduced milk.

10. Milk product comprising raw or pasteurized fat-reduced milk and tyrosinase, and having a tyrosinase modified structure.

11. The milk product of claim 10, which is an acidified milk product.
Figure 5a

Max. compression force [g]

- No enzyme: 62 g
- TG: 73 g
- TYR: 323 g

Figure 5b

Released liquid [%]

- No enzyme: 6.6%
- TG: 7.3%
- TYR: 0.0%
### A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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)

IPC8: A23C

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

FI, SE, NO, DK

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

EPO-Internal, WPI, BIOSIS, FSTA, FROSTI, CAPLUS

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
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<tr>
<td>X</td>
<td>WO 0110232 A1 (FRIESLAND BRANDS BV et al.) 15 February 2001 (15.02.2001), page 5, lines 3-10; examples; claims</td>
<td>1 - 7, 9 - 11</td>
</tr>
<tr>
<td>Y</td>
<td>US 2002061358 A1 (MIWA NORIKO et al.) 23 May 2002 (23.05.2002), abstract; examples 3, 4; claims cited in the application</td>
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**X** Further documents are listed in the continuation of Box C.  
**X** See patent family annex.

- **A** document defining the general state of the art which is not considered to be of particular relevance.
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Date of the actual completion of the international search: 10 September 2007 (10.09.2007)  
Date of mailing of the international search report: 04 October 2007 (04.10.2007)

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### CLASSIFICATION OF SUBJECT MATTER

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