

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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Me refiero al Oficio N° 4234 contentivo del concepto técnico N° 595 notificado en este asunto y al respecto me permito presentar un nuevo pliego reivindicatorio de nueve reivindicaciones.

En la Reivindicación 1, como se puede apreciar el proceso para la incorporación de proteínas de suero lácteo en un producto alimenticio, lo cual elimina la falsa dependencia con respecto a las Reivindicaciones dependientes.

La Reivindicación 3 estudiada ha sido incorporada dentro del proceso de la nueva Reivindicación 1.

Los solicitantes creen que la nueva Reivindicación independiente, así como las dependientes cumplen con los requisitos sugeridos por el Examinador.

Por otra parte, el Examinador indica que todas las etapas del proceso Reivindicado, especialmente las Reivindicaciones 1 y 2, están anticipadas por la patente de Gran Bretaña N° 1440182 de Van Pelt y otros.

Los solicitantes señalan que dicha patente está dirigida a emulsiones de aceite en agua conteniendo grasa, emulsificantes y proteína en suero complementada con un polisacárido aniónico utilizado para hacer crema batida con bajas calorías. Van Pelt y otros no enseña el método reivindicado. Van Pelt se preocupa con la formación y el mantenimiento de un agente de floculación para el emulsificador porque la formación de buena espuma solo puede ser obtenida si un floculador se encuentra presente. La invención reivindicada específicamente evita la formación de agentes floculantes durante la acidificación. (Solicitud publicada en la página 2, párrafo (0034)).

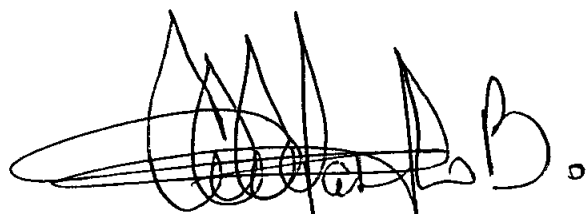
No solamente Van pelt está enfocado en la formación de espuma. Van pelt y otros enseña que el pH debe estar en el vecindario del punto isoeléctrico de la proteína, la cual Van Pelt identifica con un rango muy específico de pH 4.2 a 5.5. porque el agente floculante necesario para la formación de bastante espuma no se encuentra dispersa en una solución proteica en ese rango de pH. Van Pelt y otros no enseña que el pH debería estar por debajo del punto isoeléctrico de la proteína en suero, tal y como se reivindica. Por el contrario, Van Pelt específicamente enseña que una proteína ionizada "conteniendo un pH lejos del punto isoeléctrico" dispersará el agente floculante y la formación de espuma no se dará. (Página 2, líneas 68-72). Mientras Van Pelt y otros enseña un ajuste en el pH a pH3 en el ejemplo 1, este paso de acidificación es usado para precipitar el polisacárido y los complejos de la proteína en suero, un paso innecesario en la invención reivindicada.

Adicionalmente, el paso de pasteurización de Van Pelt y otros es mucho más bajo que el paso de calentamiento de la invención reivindicada. La pasteurización generalmente ocurre en temperaturas cercanas a 72 grados celsius, no por encima de 80 grados celsius como se reivindica. El proceso de pasteurización de Van Pelt no sería suficiente para modificar las ya modificadas proteínas en suero (debido al proceso de acidificación por debajo del pH isoeléctrico) para hacerlos más funcionales.

Más aún, Van Pelt y otros no enseña o sugiere el tratamiento (mezcla e incubación) del producto alimenticio resultante con una transglutaminasa.

En los términos anteriores doy respuesta al concepto técnico 595, por lo que pido se continúe con el trámite respectivo.

Atentamente,



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REIVINDICACIONES

1- Un proceso para la incorporación de proteínas de suero lácteo en un producto alimenticio caracterizado por:

- la acidificación de una solución acuosa de una o más proteínas de suero lácteo por debajo de su PH isoelectrico $[pI]$ a un PH en el rango de 4.5 a 2.5;
- el tratamiento térmico de dicha solución acidificada a una temperatura de más de 80°C,
- mezclando la solución acidificada de una o más proteínas de suero lácteo con una base de un producto alimenticio para formar un producto alimenticio, y
- la mezcla posterior e incubación del producto alimenticio resultante con una transglutaminasa.

2- El proceso descrito en la reivindicación 1, en el cual la relación peso de proteínas de suero lácteo a peso de grasas está comprendida dentro del rango de 3:1 a 1:5 con base en materia seca.

3- El proceso descrito en una cualquiera de las reivindicaciones 1 a 2 caracterizado porque la base de producto alimenticio contiene caseína.

4- El proceso descrito en la reivindicación 3, caracterizado porque la relación peso de proteínas de suero lácteo a peso de caseína en el producto alimenticio resultante que contiene caseína esta comprendida dentro del rango de >10:90 a 80:20, preferiblemente de 20:80 a 40:60.

5- El proceso descrito en una cualquiera de las reivindicaciones 1 a 4, caracterizado porque la base de producto alimenticio es una formulación de queso procesado.

6- El proceso descrito en una cualquiera de las reivindicaciones 1 y 2, caracterizado porque la base de producto alimenticio contiene proteínas cárnicas.

7- El proceso descrito en la reivindicación 6, en el cual la relación peso de proteína de suero lácteo a peso de proteínas cárnicas en el producto alimenticio resultante que contiene proteínas cárnicas está comprendida dentro del rango de 10:90 a 80:20, preferiblemente de 20:80 a 40:60.

- 8- El proceso descrito en las reivindicaciones 6 y 7 caracterizado porque el producto alimenticio resultante que contiene proteínas cárnicas incluye grasa animal.
9. El proceso descrito en una cualquiera de las reivindicaciones 6 a 8 caracterizado porque la base de producto alimenticio es base de carne procesada.

United States Patent [19]

Kuipers et al.

[11]

4,188,411

[45]

Feb. 12, 1980

[54] **METHOD OF PREPARING A
PROTEIN-ENRICHED UNRIPENED
CHEESE COMPOSITION**

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[21] Appl. No.: **652,181**

[22] Filed: **Jan. 26, 1976**

[30] **Foreign Application Priority Data**

Jan. 30, 1975 [DE] Fed. Rep. of Germany 2503840

[51] Int. Cl.² **A23C 19/02; A23C 19/12;
A23C 21/00**

[52] U.S. Cl. **426/582; 426/36;
426/583**

[58] Field of Search 426/36, 582, 583, 40,
426/38, 41; 260/122

[56]

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Primary Examiner—David M. Naff

Attorney, Agent, or Firm—Toren, McGeedy and Stanger

[57]

ABSTRACT

A protein-enriched, unripened cheese composition is prepared by dispersing a water-soluble concentrate of whey protein in unripened cheese.

7 Claims, No Drawings

METHOD OF PREPARING A PROTEIN-ENRICHED UNRIPENED CHEESE COMPOSITION

This invention relates to a method of preparing an unripened cheese composition enriched with whey protein.

Four methods of preparing unripened cheese compositions enriched with whey protein were known heretofore. In one method, whey protein or whey protein concentrate is added to milk, and the protein-enriched milk so obtained is caused to curdle. The curds are separated from an aqueous liquid in which some whey protein is again lost. The increase in protein content that can be achieved by this method is inherently limited.

In the second method, milk is heated to a high temperature in the presence of calcium ions prior to coagulation so that whey protein is bound to the casein and is recovered with the casein after coagulation. This method does not permit the recovery of the entire whey protein originally present in the milk, and some whey protein is inherently lost when the curds are separated from the aqueous liquid. The heating of the milk prior to coagulation adds an additional step to the normal cheese making process and consumes energy.

According to the third method, the milk is subjected to ultrafiltration, and enough water is removed thereby to avoid the formation of a separate aqueous phase or whey after coagulation. No more whey protein can be found in the ultimate product than was initially present in the milk, and ultrafiltration of the entire milk supply is costly.

The fourth method calls for thermal precipitation of protein from the whey under conditions of time and temperature to cause denaturing of the protein which thereafter no longer is soluble. This product, when mixed with unripened cheese, gives the mixture a grainy texture and a taste significantly different from that of ordinary unripened cheese. About one third of the whey protein is not capable of being precipitated by a practical heat treatment.

It is a primary object of the invention to provide a method of making an unripened cheese product enriched with whey protein which is free from the shortcomings of the afore-described known methods. It is a more specific object to provide a method which does not require significant changes in the usual procedure of preparing unripened cheese, yet permits the whey protein content of the ultimate cheese composition to be raised to any desired value, more specifically, a whey protein content higher than that of the milk from which the casein in the composition is derived. It is a further object of the invention to produce unripened cheese of low fat content having the taste and mouth feeling of a cheese rich in fat.

With these and other objects in view, as will hereinafter become apparent, the invention proposes to make an unripened cheese composition enriched with water-soluble protein by dispersing a water-soluble concentrate of whey protein in unripened cheese, itself produced in any known manner. The term "unripened cheese" is employed herein in the conventional manner to designate curds precipitated from whole milk, skim milk, or enriched milk, but not cured or ripened. Cottage cheese, German Quarg, French Gervais and Petit Suisse are typical unripened cheeses.

The term "concentrate of whey protein" or "whey protein concentrate" is employed herein to designate a composition whose solids contain a higher percentage of whey protein than the whey obtained by conventional coagulation of milk and removal of the curds. A typical whey protein concentrate of the invention contains solids consisting of 30% to 80% whey protein, preferably $\frac{1}{2}$ or more of the protein being present in soluble form. The soluble protein fraction is determined by centrifuging an aqueous medium containing 10% of the solids at 196 g. g being the normal value of terrestrial gravity. The soluble protein in the whey protein concentrate of the invention is predominantly in the native state, that is, not denatured.

Concentrates of soluble whey protein are known in themselves, for example, from the commonly owned U.S. Pat. No. 3,930,039 which involves ultrafiltration. Other methods for preparing whey protein concentrates rely on electrodialysis or molecular sieves. Such concentrates may also be prepared from whey by first removing the milk sugar, thereafter the inorganic ions by ion exchangers or by electrodialysis, and ultimately a portion of the water.

Whey protein concentrate, in the method of this invention, may be dispersed in unripened cheese as a solid powder or as a mixture with an aqueous medium having the consistency and appearance of a viscous solution. Such a fluid mixture may be prepared by combining the protein powder with water, or it may be obtained directly by any one of the methods mentioned above.

When the whey protein concentrate is employed in the method of the invention as a fluid capable of being pumped, separation of the fluid from the curds of the unripened cheese must be avoided because whey protein would be lost thereby. Phase separation can generally be avoided by maintaining a relatively high viscosity. It has been found that a fluid whey protein concentrate which would normally tend to separate from the curd because of a relatively low concentration of solids and correspondingly low viscosity can be stabilized by heating it to an elevated temperature until an adequate increase in viscosity occurs. The pH value of the concentrate during heating should be lower than its isoelectric point, but not lower than 2.5. The preferred pH range is between 2.7 and 3.7. Suitable methods of pH adjustment have been described in the abovementioned patent, but also other known methods are suitable.

The time required for achieving the desired increase in viscosity is usually between 2 minutes and 20 minutes and depends on the temperature. The temperature should be above 65° C., temperatures of 80° to 95° C. being preferred. The treatment is beneficial, for example, for whey protein concentrates containing 15%-22% of which 75% is protein or for whey protein concentrates containing 16%-24% solids of which 60% is protein, all percentage values in this specification and the appended claims being by weight unless stated otherwise.

The concentrate whose viscosity was increased by the heat treatment may be dispersed in unripened cheese without further preparatory treatment, or it may be adjusted to a higher pH, such as 3.5-5.5, before being mixed with the cheese. Preferred neutralizing agents are the salts of weak acids normally present in milk with anions also normally found in milk and include the citrates of alkali metals and alkaline earth metals, as well as neutral whey or whey concentrate. Best cheese compositions are generally obtained if the thermally treated

whey protein concentrate is adjusted to a pH value of 3.5-4.5 at which it shows a distinct maximum in viscosity. This maximum viscosity can be achieved either by adjusting the pH to a value between approximately 3.5 and the isoelectric point prior to heating, or by adjusting to a lower pH prior to heating, as mentioned above, and then adjusting to a pH between 3.5 and 4.5.

Typically, a whey protein concentrate containing 15% solids and having a viscosity of about 100 centipoises at 20° C. may be given a viscosity of more than 1000 centipoises at 20° C. by a one-minute heat treatment at 97° C. and pH 3 followed by pH adjustment to 3.7. Surprisingly, no coagulation occurs during this treatment.

While it is important that most of the whey protein, preferably 3 or more, which is present in the concentrate be soluble in water prior to heating in an acidic medium, the solubility of the whey protein after this treatment is less critical.

Whey protein concentrate in the form of a dry powder is conveniently stored. In a preferred embodiment of the invention, the powder is dispersed in water, and the viscosity of the resulting mixture is raised by heating at a pH below the isoelectric point of the whey protein as described above. The fluid concentrate so obtained has the approximate consistency of a custard and often is thixotropic. When it is dispersed in unripened cheese, there is obtained a composition free from ingredients which would not be present in whole milk. No synthetic or other extraneous stabilizers are present, and none are needed.

It is preferred that the whey protein concentrate have a solids content approximately equal to that of the unripened cheese. This is not usually possible without the acidic thermal pre-treatment of the concentrate. Skim milk curds, for example, have a normal solids content of 18%. A whey protein concentrate of the same solids content cannot be used to best advantage without the pre-treatment. An ordinary whey protein concentrate containing 18% solids can be dispersed in the curds, but the mixture would soon release a thin liquid and may not be salable. When the same concentrate is heated to increase its viscosity, as described above, the cheese composition obtained by dispersing the concentrate in the unripened cheese is stable in storage and shows less tendency to spoil than the unmixed unripened cheese.

The ratio of casein to whey protein in whole cow's milk is approximately 6:1. The same ratio is easily established in an unripened cheese composition according to the invention. The solids in skim milk curds, for example, contain 75% casein. A corresponding whey protein concentration of 75% may be achieved in the solids of a whey protein concentrate. The two components thus need be mixed only in the ratio of their solids contents. Obviously, an unripened cheese composition containing whey protein in a higher ratio relative to the casein present may be prepared without difficulty.

The method of the invention does not require any changes in the conventional production of the unripened cheese, yet permits the making of a cheese composition having virtually any desired content of whey protein and a correspondingly enhanced nutritive value. The cheese compositions of the invention are generally preferred to the unripened cheese base because of superior taste and consistency. A low-fat cheese composition of the invention has the taste and texture of an unripened cheese much higher in fat content. In organoleptic tests, a cheese composition pre-

pared by the method of the invention and containing 0-2% fat, based on total solids, was considered the equivalent of a conventional unripened cheese containing 10% to 40% fat. The compositions of the invention can be prepared from dry whey protein concentrate stored in the form of powder over extended periods without impairing the taste of the composition.

The following Examples are further illustrative of this invention.

EXAMPLE 1

A spray-dried whey protein concentrate was prepared by ultrafiltration as described in the aforementioned patent to contain approximately 1-3% water, 4-6% fat, 70-80% protein, 5-15% lactose, and 2.5% inorganic matter after adjustment to pH 2.7-3.7. More than 90% of the powder dissolved in water.

0.9 kg Whey protein powder was dispersed in 8.2 kg water, and the fluid mixture was found to have a pH value of 3.5. It was held at 95° C., cooled below 60° C., and combined with 0.9 kg of a similarly produced whey protein powder having a pH of 6.2-6.7. The mixture so prepared was sterilized at 70° C. for two minutes and had then a pH of 4.9.

1.75 kg of this material was mixed with 10 kg skim milk curds containing 18% solids by stirring at 1500 r.p.m. for 30 seconds. The product so obtained had a pH value of 4.65-4.50 and the taste and consistency of curds prepared from milk enriched with cream, similar to cream cheese. It was very different from the skim milk curds employed which had a somewhat grainy texture reminiscent of straw in that they gave a gentle scratching sensation in the throat when swallowed. These curds which were employed throughout the following Examples had a pH of 4.6.

EXAMPLE 2

In a procedure otherwise identical with that of Example 1, the amount of concentrate powder subjected to thermal treatment was increased to 1.35 kg, while the neutral whey protein powder was reduced to 0.45 kg. The period of heat treatment was shortened to ten minutes. The whey protein concentrate had a pH value of 4.2 prior to mixing with curds, and the cheese composition ultimately produced had a pH of 4.55-4.45. It was somewhat firmer than the product made in Example 1, but was not significantly different in taste.

EXAMPLE 3

1.8 kg Spray-dried whey protein concentrate of the composition indicated in Example 1 and having a pH of 3.5 was dispersed in 8.2 kg water, and the mixture was kept at 90° C. for 5 minutes. It was then dispersed in 10 kg skim milk curds as in Example 1 to produce a cheese composition having the same consistency or texture as the product of Example 2, but a slightly more acidic taste at pH 4.45.

EXAMPLE 4

A whey protein powder nominally containing 1-3% water, 4-6% fat, 55-65% protein, 20-30% lactose, 3-5% mineral matter, having a pH of 2.7-3.7 and a water solubility greater than 90%, was mixed with water in a ratio of 1.8 kg to 8.2 kg. The mixture, which had a pH of 3.4, was held at 80° C. for two minutes.

The thickened, fluid whey concentrate so prepared was mixed with 10 kg skim milk curds in an amount of

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2 kg as in the preceding Examples, and a smooth cheese composition of excellent taste was obtained.

When only 1.0 kg of the same whey protein powder was dispersed in 8.2 kg water and subjected to heat treatment at 85° C. for 5 minutes, and further mixed with 0.8 kg whey protein powder of pH 5.9 which differed from the above composition by a correspondingly higher content of mineral matter (5-8%), a whey protein concentrate of lower viscosity having a pH value of 4.5 was obtained. Yet, it yielded a mild and smooth, stable cheese composition when combined with skim milk curds in the manner described above.

EXAMPLE 5

1.3 kg Whey protein powder of the composition given in Example 1 (pH 3.4) was dispersed in 8.7 kg whey of pH 4.5, and the mixture was held at 90° C. for 5 minutes, whereby a concentrate of pH 3.95 was obtained, but was grainy and unsuited for mixing with unripened cheese to the excessively high pH of the mixture subjected to the heat treatment.

When the same amount of the whey protein powder described in Example 1 (pH 2.5) was dispersed in the whey in the same ratio, and the mixture (pH 3.2) was held at 97° C. for two minutes, there was obtained a smooth, very viscous fluid which was dispersed in skim milk curds in a ratio of 2 kg/10 kg as in Example 4 to prepare a cheese composition which was smooth, aromatic, and slightly acidic (pH 4.45).

EXAMPLE 6

The whey protein powder of pH 3.4 described in Example 4 was dispersed in water in a ratio of 1.2 kg to 8.2 kg, and the fluid mixture was held at 90° C. for four minutes. It was thereafter mixed with 0.6 kg of the more neutral whey powder described in Example 4 (pH 5.9). The mixture then had a pH of 4.25 and was just sufficiently fluid to be pumped. It was mixed with skim milk curds as in Example 4 and a smooth cheese composition having a somewhat bland taste was obtained.

When a batch of whey protein concentrate prepared in the same manner was mixed with skim milk curds in a ratio of 10 kg to 100 kg by means of a continuously operating pump, the resulting cheese composition had a pH of 4.5 and was smooth, somewhat soft, but of pleasant, cream-like taste.

EXAMPLE 7

1.5 kg Whey protein powder of the composition given in Example 1 and having a pH of 3.6 was dispersed in 11.3 kg water, and the mixture was heat-treated at a temperature gradually increasing from 60° to 90° C. in 20 minutes. Thereafter, 1.2 kg of the same powder was mixed in, and a very viscous material was obtained.

It was mixed with skim milk curds in a ratio of 25 kg to 150 kg for 7 minutes by means of a pump. The cheese composition so prepared had a pH of 4.5, a smooth, somewhat fluid texture, and a pure, acidic taste.

EXAMPLE 8

10 kg Whey protein powder as in Example 4 (pH 3.4) was combined with 55 kg water, and the mixture was held at 92° C. for five minutes, whereupon it was further mixed with 4.2 kg whey protein powder of similar composition, but pH 5.9. A creamy product having a pH of 4.0 was obtained.

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100 kg Skim milk curds were continuously mixed with 20 kg of the product by means of a pump and a pleasantly smooth cheese composition of creamy taste and pH 4.5 was formed.

EXAMPLE 9

2.5 kg Whey protein powder of a composition as in Example 4, but having a pH of 3.6, was dispersed in 7.5 kg water, and a smooth, somewhat viscous whey protein concentrate was obtained. It was dispersed in skim milk curds at a ratio of 2 kg to 10 kg by stirring as in Example 1. The cheese composition so prepared had a pH of 4.45 and was of smooth texture and pleasant, cream-like taste.

A closely similar cheese composition was obtained in an otherwise identical procedure starting with the whey protein powder of Example 1 at pH 3.6.

EXAMPLE 10

Four batches of the whey protein powder described in Example 4 (pH 3.4) were dispersed in water in a ratio of 1.35 kg to 6.5 kg, and the four mixtures were adjusted to respective pH values of 2.7, 3.0, 3.3, and 3.7. Each batch was held at 95° C. for five minutes, cooled to 55° C., mixed with 0.45 kg of otherwise similar whey protein powder of pH 6.5, and the concentrates so obtained, which were viscous, but capable of being pumped, had pH values of 3.73, 3.85, 4.00, and 4.30 respectively. They were mixed with skim milk curds at a weight ratio of 1:5 to produce four equally acceptable cheese compositions varying in pH value from 4.40 to 4.45.

EXAMPLE 11

300 g Whey protein powder of the composition given in Example 4 and having a pH of 3.7 was stirred into water to produce a mixture containing 17% solids which was then held at 90° C. for two minutes. The fluid so obtained had a cream-like consistency, and its pH value was adjusted to 4.0 by the addition of 3.8 g trisodium citrate. This concentrate was dispersed in skim milk curds as described in the preceding examples to yield cheese compositions of excellent taste and texture.

Analogous results were achieved when the aqueous dispersion of the whey solids powder was adjusted to pH 3.2 prior to heating and to pH 4.0 by means of 10 g sodium citrate after heating and cooling.

EXAMPLE 12

1000 kg Skim milk was curdled as is conventional in the making of cottage cheese. Instead of the cream normally employed in preparing creamed cottage cheese, 40 kg of a 20% whey protein concentrate, prepared as in Example 6, was intimately mixed with the curds. The cheese composition so obtained was hardly distinguishable from creamed cottage cheese prepared by good, conventional practice.

When skim milk curds described in the preceding Examples were replaced by curds containing substantial amounts of butter fat derived from the whole milk from which the curds were precipitated or otherwise mixed with the casein, correspondingly enriched unripened cheese compositions were obtained and were of pleasing taste and textures. The effects of the whey protein concentrates, however, are demonstrated most strikingly by the improvement which they contribute to a low-fat composition.

It should be understood, therefore, that the foregoing disclosure relates only to preferred embodiments of the invention, and that it is intended to cover all changes and modifications of the Examples of the invention herein chosen for the purpose of the disclosure which do not depart from the spirit and scope of the invention set forth in the appended claims.

What is claimed is:

1. A method of making an unripened cheese which comprises: (a) adjusting a fluid, water-bearing concentrate of water-soluble whey protein to a pH value between 2.5 and 3.7, said value being below the isoelectric point of said whey protein; (b) heating the adjusted concentrate to an elevated temperature for a period sufficient to increase the viscosity thereof; and (c) dispersing the fluid concentrate of increased viscosity in unripened cheese.

2. A method as set forth in claim 1, wherein said temperature is above 65° C.

3. A method as set forth in claim 2, wherein the solids content of said fluid is approximately equal to the solids content of said unripened cheese.

4. A method as set forth in claim 2, wherein said temperature is 80° to 95° C.

5. A method as set forth in claim 4, wherein said concentrate of increased viscosity is adjusted to a pH value of 3.5 to 5.5 before being dispersed in said cheese.

6. A method as set forth in claim 5, wherein said concentrate is prepared, prior to said adjusting, by ultrafiltration of whey.

7. A method as set forth in claim 6, wherein said whey, prior to said ultrafiltration, is adjusted to a pH value lower than the isoelectric point of said whey.

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US005834232A

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United States Patent [19]

Bishop et al.

[11] Patent Number: 5,834,232
[45] Date of Patent: Nov. 10, 1998

[54] CROSS-LINKED GELATIN GELS AND METHODS OF MAKING THEM

93/22930 11/1993 WIPO

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[75] Inventors: Paul D. Bishop, Fall City; Gerald Lasser, Everett, both of Wash.

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[73] Assignee: ZymoGenetics, Inc., Seattle, Wash.

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[21] Appl. No.: 641,463

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[22] Filed: May 1, 1996

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[51] Int. Cl.⁶ C12P 21/06; C12N 11/04; C12N 9/10; A61K 38/17

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[58] Field of Search 435/68.1, 174, 435/177, 180, 182, 193; 530/356, 354

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Primary Examiner—Jean C. Witz

Assistant Examiner—Susan Hanley

Attorney, Agent, or Firm—Gary E. Parker; Paul G. Lunn

[57]

ABSTRACT

Enzymatically cross-linked protein gels and methods for preparing them are disclosed. The methods comprise adding a transglutaminase, such as factor XIII, to a composition of a temperature-sensitive gel-forming protein, such as gelatin or collagen, and incubating the composition and transglutaminase under gel-forming conditions. The resulting gels have superior strength and thermal stability, and can be used within a variety of medical and industrial applications.

28 Claims, 2 Drawing Sheets

linked and non-cross-linked gels. Such applications include photographic films, gelatin-containing foodstuffs, gelatin capsules (particularly microcapsules), drug delivery devices, and prosthetic devices. The enzymatically cross-linked gels of the present invention provide the advantages of uniformity and specific cross-linking in combination with high thermal stability, which permit use of the gels within new and expanded applications.

The methods of the present invention can be used in the preparation of photographic emulsions. Photographic emulsions are typically gelatin-based emulsions containing one or more of silver halide grains, dye-forming coupler compounds, ultraviolet absorbing materials, developing agents, etc. These compounds are added to the gelatin to form a mixture which is applied to a polymeric film base or paper surface in the sol state and allowed to gel and cross link. Surfaces can be coated with single or multiple layers of emulsion as desired. Methods for preparing photographic emulsions and applying them to surfaces are known in the art. See, for example, U.S. Pat. Nos. 3,607,345; 4,214,047; and 4,269,927, which are incorporated herein by reference in their entirety.

The methods of the present invention can also be used in the preparation of protein-containing food products, for example to improve the functional properties of proteinaceous foods. Within the present invention, transglutaminase is added to such foodstuffs as minced meat, fish paste, milk, gelatin, whey protein, soy protein, wheat protein, maize protein, egg albumin, rape seed protein, potato protein, and the like. The resulting mixture is incubated for a period of time sufficient for gelation to occur and for the transglutaminase to react with the substrate. Foodstuffs prepared in this manner exhibit increased water binding capacity, improved texture, improved thermal stability, and other advantages. The resulting alteration in the functional properties can be utilized to lower the fat content in the food product, because the crosslinked protein simulates properties associated with a higher fat content. The methods of the present invention are particularly useful in the preparation of food products rich in gelatin or collagen, such as gelatin desserts and collagen sausage casings.

The methods of the present invention can be used to prepare drug delivery vehicles such as microcapsules. For example, gelatin microcapsules can be prepared by complete coacervation (Thies, *Crit. Rev. Biomedical Engineering* 8: 335-383, 1982; incorporated herein by reference). Two oppositely charged polyelectrolytes (gelatins type A and B) are combined in an aqueous solution to form a coacervate which also includes the drug to be encapsulated. During gelation, the mixture is vigorously agitated to form small beads, which are cross-linked by transglutaminase as disclosed herein, making them relatively insoluble, yet biodegradable. In the alternative, microcapsules can be formed from organic/aqueous emulsions according to known methods and subsequently cross-linked. Microcapsules containing both peptidic and non-peptidic drugs can be prepared by this method. In a related embodiment, drugs containing amines, including proteins and peptides containing lysine residues, can be covalently bonded to microcapsules during the hardening process. Amine-containing substances can be added to the gel prior to or during cross-linking, whereby the amines become covalently bonded to glutamine residues in the gel-forming protein by the action of the transglutaminase. Microcapsules of this type can be used to provide localized or timed-release delivery of drugs. Drugs that can be delivered in this way include hirudin, glucagon, insulin, protamine, amphotycin, and bacitracin.

The gels of the present invention can also be used in prosthetic devices, for example to promote the adhesion of vascular endothelial cells seeded on prosthetic vascular grafts. Methods for seeding and sodding vascular grafts with endothelial cells are disclosed by, for example, Budd et al., *Br. J. Surg.* 76:1259-1261, 1989; Kaehler et al., *J. Vasc. Surg.* 9:535-541, 1989; Mosquera and Goldman, *Br. J. Surg.* 78:656-660, 1991; WIPO Publication WO 91/16009; and Rubens et al., U.S. Pat. No. 5,324,647. Vascular endothelial cells attach and spread when their cell membrane integrins is contact and bind to matrix components such as fibronectin, laminin, and von Willebrand factor. In the case of fibronectin the N-terminus contains a transglutaminase substrate site that allows it to be cross-linked to a gelatin or collagen matrix. See Bockenstedt et al., *J. Clin. Invest.* 78:551-556, 1986. The C-terminal portion of fibronectin contains the ligand for binding to the $\alpha 5 \beta 1$ integrin. Properly oriented fibronectin will act as a tether for endothelial cells and could be used to reduce sluffing of seeded endothelial cells. Other proteins that provide contact points for cell attachment and/or migration and cytokines can also be included. Polyamines, such as spermidine, cadaverine, and putrescine can also be included to provide cell-attachment points.

Protein gels can be shaped by casting (forming the gel within a mold while in a plastic state) or by extruding during or after cross-linking to produce a fiber that can be woven. Additional compounds can be incorporated into or onto such gels. For example, amine-containing compounds can be bound to a surface of a prosthetic material to provide a transglutaminase substrate site. Methods of binding include treatment with a strong oxidant, such as potassium permanganate, nitric acid, dichromates, or peroxides; cross-linking; and adsorption.

Cross-linked gels of the present invention can be used in surgical applications where non-cross-linked gels are currently used, such as in neural reconnection surgery to prevent adhesions. Polymeric gels of this type are known in the art (e.g., ADCON®, semisynthetic carbohydrate polymeric gels, produced by Gliatech Inc., Cleveland, Ohio). Cross-linking stabilizes the gel, increasing its residence time.

Enzymatically cross-linked gels are particularly well suited for in vivo uses in view of the protease resistance of stabilized helices and the improved thermal stability of the gels. Gels having a melting point of 37° C. or above can be prepared, with the actual melting point depending on the degree of cross-linking. It is thus possible to prepare gels that are stable at, for example, 45° C., 65° C., or 100° C.

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

EXAMPLES

Example 1

All viscosity measurements were taken at 37° C. in a jacketed Brookfield DV III cone and plate viscometer with a cp40 one (Brookfield Engineering Labs, Stoughton, Mass.).

Human factor XIII A₂ dimer was produced in *S. cerevisiae* and stored as a lyophilized powder containing 13.2 mg factor XIII, 0.66 μ Moles EDTA, 6.60 μ Moles glycine, and 0.13 grams sucrose. Prior to use, the lyophilized material was dissolved in 1 ml of distilled water.

Calf skin Type A gelatin, high Bloom strength (Ferrosan International A/S, Soeborg, Denmark) was dissolved in

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DIVISIÓN DE NUEVAS CREACIONES

2020

Abogado
JERONIMO CASTILLO BONILLA

ASUNTO Radicación 03-98070
 Trámite 011
 Evento 379
 Actuación 430
 Oficio No 9 4 9 1
 Folios 011

Patente de Invención ☒ Patente de Modelo de Utilidad ☐
Vía Nacional ☐
Vía PCT (fase nacional) ☒ Cap. I ☐ Cap. II ☒

No. Sol. Internacional: } PCT/US02/10887
Fecha de Presentación Internacional: abril 08 de 2002

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

- 1.1. Descripción: Folios: 30 a 37
1.2. Reivindicaciones: Folios: 38 a 39 (como se radicó inicialmente)
 Folios: 79 a 80 (modificadas)
 Folios: 91 a 92 (modificadas)
1.3. Prioridad: 09 de abril de 2001, EP. No. 011078524

2. Objeto de la invención

- La Invención se titula **“PROCESO PARA LA PREPARACIÓN DE UNA EMULSIÓN GRASA ESTABILIZADA CON PROTEINAS DE SUERO LÁCTEO”** (folio 78)

La **reivindicación independiente 1** define un “proceso para la incorporación de proteínas de suero lácteo en un producto alimenticio caracterizado por:

- > la acidificación de una solución acuosa de una o más proteínas de suero lácteo por debajo de su pH isoelectrico a un pH en el rango de 4.5 a 2.5
- > el tratamiento térmico de dicha solución acidificada a una temperatura de más de 80°C,
- > mezclando la solución acidificada de una o más proteínas de suero lácteo con una base de un producto alimenticio para formar un producto alimenticio, y
- > la mezcla posterior e incubación del producto alimenticio resultante con una transglutaminasa.

- El problema planteado en esta solicitud consiste en desarrollar un proceso para incorporar una mayor cantidad de proteínas de suero lácteo en un producto alimenticio.

La solicitud en estudio presenta un proceso para preparar incorporar proteínas de suero lácteo en un producto alimenticio, el cual comprende acidificar una solución acuosa que contiene proteínas de suero lácteo; realizar un tratamiento térmico a dicha solución acidificada; mezclar la solución acuosa acidificada tratada térmicamente con una base de producto alimenticio y la posterior mezcla e incubación del producto resultante con una enzima.

- El objeto de la invención se catalogó como A23J 3/08 según la Clasificación Internacional de Patentes (IPC).

3. De la solicitud de Patente (según el caso)

3.1. Reivindicaciones (artículo 30 D 486)

- La reivindicación 2 señala un proceso de acuerdo con la reivindicación 1 en donde la relación peso de proteínas de suero lácteo a peso de grasas está comprendida dentro del rango de 3:1 a 1:5 con base en materia seca. Sin embargo, la reivindicación 1 no menciona dichas grasas, por lo cual existe una falsa dependencia. Es importante aclarar que la reivindicación dependiente es aquella que hace referencia a una anterior y contiene todas las características de aquella y solo puede referirse a características particulares de un elemento de la reivindicación independiente. En este caso, la reivindicación 2 adiciona elementos no contemplados en la reivindicación inicial, lo cual es inaceptable.

Así las cosas, la reivindicación 2 debe ser eliminada del capítulo en cuestión.

En conclusión, el capítulo reivindicatorio no cumpliría con el requisito de claridad de acuerdo con el artículo 30 de la Decisión 486.

4. Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 09 de abril de 2001, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a esta.

Nº	Documento	Título	Fecha de publicación
D2	US 4,188,411	"METHOD OF PREPARING A PROTEIN-ENRICHED UNRIPENED CHEESE COMPOSITION"	12 de febrero de 1980
D3	US 5,834,232	"CROSS-LINKED GELATIN GELS AND METHODS OF MAKING THEM"	10 de noviembre de 1998

5. Análisis de patentabilidad (según requisito) (artículo 14 D486)

Es importante mencionar, que la materia de invención de la solicitud en estudio, señalada en las reivindicaciones 1 a 8 del capítulo reivindicatorio modificado, corresponde a la misma materia de invención definida en las reivindicaciones 5 a 13 del capítulo reivindicatorio inicialmente presentado, la cual ya presenta un análisis de patentabilidad contenido en el pasado concepto técnico No. 1723.

5.1 Nivel inventivo (artículo 18 D486)

Respecto al estado de la técnica

- El documento US 4,188,411 (llamado D1 de aquí en adelante), divulga un método para preparar un producto alimenticio (composición de queso sin madurar) que contiene proteína de suero lácteo, el cual consiste de las siguientes etapas:
 - Ajustar un fluido, concentrado acuoso de la proteína soluble en agua del suero lácteo a un valor de pH entre 2.5 a 3.7 por debajo del punto isoeléctrico de dicha proteína de suero,
 - Calentar el concentrado ajustado a una temperatura elevada, es decir, por encima de 65°C, como por ejemplo de 80°C a 95°C, por un período suficiente para incrementar la viscosidad; y
 - Dispersar el concentrado fluido de viscosidad incrementada (solución acidificada de proteínas) en una base de producto alimenticio para formar un producto alimenticio (queso sin madurar) con un alto contenido de proteína de suero y valor nutritivo.
(folio 94, columna 1, líneas 6-8, líneas 44-47; folio 94, columna 2, líneas 24-27, 42-45, 50-51; folio 95, columna 3, líneas 47-57; folios 95 y 96, ejemplos; folio 97, reivindicaciones 1 y 4)
- El documento US 5,834,232 (llamado D3 de aquí en adelante), divulga un procedimiento para mejorar las propiedades funcionales de productos alimenticios que contienen proteínas, el cual comprende la adición y mezcla de una transglutaminasa con un producto alimenticio que contiene proteínas y la incubación de la mezcla enzima/producto alimenticio mencionada anteriormente (folio 99, columna 9, líneas 23-38)

Respecto a la solicitud en estudio:

- De acuerdo con la reivindicación independiente de la solicitud en estudio, se entiende que el principio técnico sobre el cual se fundamenta la propuesta consiste en desarrollar un procedimiento para incorporar proteínas de suero lácteo en un producto alimenticio. No obstante, en D2 se encuentra divulgado un procedimiento para introducir un alto contenido de proteína de suero lácteo en un queso sin madurar (producto alimenticio).

La única diferencia entre la solicitud en estudio y D2 corresponde a la última etapa del proceso, la cual se relaciona con la mezcla e incubación del producto alimenticio con transglutaminasa. Sin embargo, D3 divulga el tratamiento enzimático de un producto alimenticio que contiene proteínas, el cual se realiza con una transglutaminasa, tal como lo indica la solicitud en estudio.

Por lo expuesto anteriormente, un técnico de nivel medio versado en la materia que tuviera acceso a D1 y D2, podría desarrollar procedimientos para incorporar proteínas de suero lácteo en un producto alimenticio a partir de la acidificación y tratamiento térmico de una solución acuosa de proteínas, la cual después se mezcla con una base alimenticia para obtener un producto alimenticio que posteriormente se somete a un tratamiento enzimático.

Dado lo anterior, la propuesta de la solicitud no representa un avance tecnológico o un salto cualitativo sobre el estado de la técnica.

En conclusión, la materia de invención de la solicitud en estudio se encuentra afectada en su nivel inventivo ya que se deriva de manera evidente del estado de la técnica y puede ser obvia para un técnico de nivel medio versado en la materia. Por tanto, no cumple con las disposiciones del artículo 18 de Decisión 486 de la Comisión de la Comunidad Andina.

6. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

N°	Documento	Reivindicaciones afectadas	Requisito que afecta
D2	US 4,188,411	1-8	Nivel Inventivo
D3	US 5,834,232	1-8	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha
18 JUL. 2008

CONCEPTO TÉCNICO No. 1718	EXAMINADOR TÉCNICO Código No. 202076
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7/12/11