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(54) **PRODUIT LAITIER ANTI-CARIOGENE ET SON UTILISATION**
(54) **ANTI-CARIOGENIC MILK PRODUCT AND USE**

(57) Utilisation d'un lait emprésuré en quantité efficace pour préparer des compositions alimentaires réfrigérées destinées à prévenir ou à traiter la carie dentaire ou la plaque dentaire et compositions ainsi préparées.

(57) The invention concerns the use of a rennet milk in sufficient amount for preparing refrigerated food compositions for preventing or treating dental caries or plaque and the resulting compositions.

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(54) Titre: PRODUIT LAITIER ANTI-CARIOGENE ET SON UTILISATION

(57) Abstract

The invention concerns the use of a rennet milk in sufficient amount for preparing refrigerated food compositions for preventing or treating dental caries or plaque and the resulting compositions.

(57) Abrégé

Utilisation d'un lait emprésuré en quantité efficace pour préparer des compositions alimentaires réfrigérées destinées à prévenir ou à traiter la carie dentaire ou la plaque dentaire et compositions ainsi préparées.

ANTICARIOGENIC DAIRY PRODUCT AND ITS USE

The present invention relates to a dairy product which possesses anticariogenic properties. The present invention also relates to the food compositions prepared from the said product.

State of the art

Some products derived from milk are of great interest for dentiboccal health. Thus, anticariogenic properties are recognized for casein in certain forms and for some of its derivatives. A few cheeses, for example, are known for their anti-caries activity (Nutrition Reviews, 46, 215-217, 1988; Pause, B. and Lembke, J., Milchwissenschaft, 47, 697-700, 1992; Pause, B. and Lembke, J., Milchwisssenschaft, 48, 137-141, 1993).

In patent US 5427769 (Nestec S.A.), the appearance of dental caries is prevented by placing in contact with the teeth a food composition containing micellar casein in a sufficient quantity to inhibit colonization by *Streptococcus sobrinus*, the principal bacterial agent involved in carious pathology.

Patent EP 748591 (Société des Produits Nestlé S.A.) also describes the use of fluorinated micellar caseins or their micellar subunits for treating plaque or dental caries.

Patent US 4992420 (Nestec S.A.) describes the treatment of the buccal cavity with κ-caseino-glycomacropeptides (CGMP) derived from whey in order to eradicate dental plaque and caries. Likewise, patent US 4994441 (Nestec S.A.) describes an anti-plaque and anti-caries composition in which the active agent is chosen from κ-caseinoglycopeptides.

Furthermore, patent US 5,015,628 (University of Melbourne) describes an anticariogenic composition containing, as active agent, in particular phosphopeptides obtained, for example, by trypsin hydrolysis of α- and β-caseins.

All these documents describe processes which do not belong to the technology of fresh dairy products. The processes currently used to manufacture such fresh products lead to acidic products, such as yogurt, fromage frais or "petit suisse". In such products, acid coagulation of casein leads to its decalcification, thus bringing about the loss of some of its anticariogenic properties. Likewise, the CGMP contained in these products is not hydrolysed, and as a result is not in an active form.

The present invention is intended to overcome these disadvantages, by providing a fresh dairy product having optimized anticariogenic properties.

Summary of the invention

The main subject of the present invention is the use of a "rennetted" milk for preparing refrigerated food compositions intended for preventing or treating dental caries or dental plaque. This rennetted milk is prepared by coagulating milk at neutral pH with rennet and then adding a calcium salt. The rennetted milk is stabilized in gel form. It comprises free CGMP and casein still complexed with calcium (calcified casein), although having lost its micellar form.

It has indeed been possible to observe that this coagulated milk no longer possesses a micellar casein, but that the latter retains nevertheless some of its properties, in particular as regards its calcification. Furthermore, as the whey is not separated from the coagulum, the CGMP is preserved with the product.

The results *in vivo* show that this milk has anticariogenic properties which are superior to those of other refrigerated dairy products such as yogurt, for example. The advantage of the invention compared with products already known is that of providing a fresh dairy product whose activity is optimized and which groups together in their active form, two anti-caries constituents of milk, namely calcified casein and CGMP.

Finally, the subject of the present invention is the food compositions produced from this rennetted milk, which is added in an effective quantity so as to obtain a fresh dairy product having an optimized anti-caries and anti-dental plaque activity. These compositions may also be provided in the form of a powder.

Detailed description

In the description, the percentages are indicated by weight relative to the total weight, unless otherwise stated.

The rennetted milk according to the present invention is used to prepare food compositions intended for preventing or treating dental caries or dental plaque. It contains caseinoglycomacropeptide (CGMP) in free, hydrolysed form and coagulated casein complexed with calcium (calcified casein).

CGMP is a glycopeptide hydrolysed from kappa-casein by the action of rennet. It is present in the coagulation whey and is not separated during the process for the preparation of the said rennetted milk. The quantity of free CGMP present in the rennetted milk is preferably between 0.05 and 0.2%, which represents 1.5 to 6% of the protein matter.

Calcified casein is also produced by the action of the rennet on the casein. It has lost its micellar form as is the case for other fresh dairy products such as yogurts or acidic cheeses, but unlike the latter, it retains some of the properties of the micelle. It remains in particular in a calcified form. The calcified casein content of the said rennetted milk is preferably greater than 2%.

The rennetted milk according to the invention is also rich in calcium. This calcium is present in 2 forms: one which is associated with casein, and the other which is free. The minimum quantity of calcium contained in the rennetted milk is 1.75 g/l.

The rennetted milk according to the invention has the characteristic of grouping together these two anti-caries compounds: CGMP and calcified casein in their active form. Tests carried out *in vivo* show that it has anticariogenic properties superior to those of other dairy products such as yogurt, for example. This surprising result is explained by a synergistic effect between CGMP in hydrolysed form and calcified casein.

It is possible to prepare a mixture containing at least 80% of milk, in the form of whole milk and/or milk enriched with fat, and 1 to 10% of powdered milk, so as to increase the dry matter content. It is possible to add potassium sorbate in an amount of about 0.1% by weight, so as to protect the product from moulds.

The whole milk and/or milk enriched with fat may be used in liquid or powdered form. Its fat content is preferably greater than 4%. A milk having a minimum dry matter content of 8% is preferably used.

Water is added to the mixture so as to obtain a paste. The paste can then be gelled by adding thereto a solution containing 10% of agar/gelatin, at a temperature of 50°C. The addition is made by dispersion at cold temperature. The gelatin may be replaced with a gum for example.

The mixture may be filtered before being pasteurized at 125°C for about 20 s. It can then be heated to 70°C before being homogenized at a pressure of about 200 bar. Next, after preliminary cooling of the mixture to around 45°C, calcium chloride in solution at 30-33% and rennet at 1/15000 are added, for example. The quantity of calcium chloride ($CaCl_2$) is preferably between 0.02 and 0.05% by weight relative to the mixture. For the rennet, it is possible to use it at 1/15000 in an amount of 0.005 to 0.008%. The mixture is then stirred. The conditions are such that the milk-based mixture does not curdle during this rennetting step. For that, the duration of homogenization of the mixture at around 45°C is preferably less than 30 min.

The mixture may then be packaged at a temperature of 43°C, for example, and then it may be kept in an oven at 43°C for 1-2 hours for curdling. A rennetted milk, coagulated at neutral pH and which still contains, at the end of the process, a quantity of free CGMP and casein complexed with calcium, is then obtained.

This process therefore makes it possible to group together and to preserve, in a simple manner, these two anticariogenic and anti-dental plaque constituents in their active form, the casein in a calcified form and the CGMP hydrolysed.

The rennetted milk enters into the preparation of various food compositions, for example gelled milk with storage lives of 2, 3 or 4 weeks. It is also possible to prepare powdered compositions, it being possible for the fresh product to be, in this case, spray-dried, for example so as to obtain it in powdered form.

These compositions contain an effective quantity of the said rennetted milk; they have an optimized anti-caries and anti-plaque activity. To treat dental caries, the minimum effective quantity of rennetted milk is preferably greater than 10%. It is possible to enhance the anti-caries activity of such compositions by adding CGMP, for example.

The compositions may be sweetened by the addition of 1 to 5% of a sweetener, preferably xylitol. It is also possible to use Aspartame, sucrose, tagatose or a maltitol/fructose mixture, for example. The compositions may also be flavoured.

Tests were carried out to compare the anticariogenic effect of the rennetted milk with that of some other fresh dairy products such as, for example, yogurt (Example 1). The results on the *in vivo* effects have shown significantly the greater efficacy of rennetted milk in anticariogenic activity.

The examples described below are not limiting and serve to illustrate the invention. The percentages and parts are indicated by weight unless otherwise stated.

Example 1 : rennetted milk

A paste is prepared by mixing with 86 kg of milk enriched with 5% of fat and having a dry matter content of 8%, 7.3 kg of powdered milk at 1% and then 120 g of potassium sorbate. The paste is gelled by adding 400 g of a solution containing 10% agar/gelatin (200°B). The addition is carried out at a temperature of 50°C and by dispersion at cold temperature. The mixture is then filtered before being pasteurized at 125°C for 20 s. It is then heated to 70°C and then homogenized at a pressure of 200 bar. The mixture is allowed to cool to a temperature of 45-46°C. It is then supplemented with 50 g of calcium chloride ($CaCl_2$) in solution at 30-33% and 8 g of rennet 1/15000. The mixture is stirred for less than 30 min at 45°C.

The mixture is then kept in an oven at 43°C for 1-2 hours. A rennetted milk, coagulated at neutral pH and which contains at the end of the process a quantity of about 0.1% of free CGMP and about 2% of casein complexed with calcium, is then obtained. This rennetted milk is used as a base in the preparation of various anticariogenic food compositions.

Example 2 : Comparison of the effects of the rennetted milk and of plain yogurt on caries in rats

3 compositions are prepared which contain no sucrose:

- a rennetted milk corresponding to the invention as prepared according to Example 1;
- a traditional plain yogurt; and

- a control based on soya bean protein (flour) (control D_0).

3 other compositions corresponding to the preceding one, supplemented with 10% sucrose, the last being the control D_1, are next prepared.

Finally, the anti-caries effect of the rennetted milk obtained according to the invention is compared with that of the traditional yogurt, both products containing or not containing 10% sucrose.

For that, 6 groups of 10 rats are prepared, which rats are infected orally twice a day by inoculating them with suspensions of *Streptococcus sobrinus* and *Actinomyces viscosus*. To promote the implantation of these bacteria, the rats are given sweetened water containing 3% of glucose and 3% of sugar for several days. These rats are then fed according to a precise programme divided into 6 distinct treatments described in Table 1. All the rats follow a "highly cariogenic" basic regime (D_2), distributed in the form of 18 daily rations. The rats are provided alternately with 18 "highly cariogenic" rations and 18 rations, either fresh dairy products described above in treatments 3, 4, 5 and 6, or controls D_0 and D_1 in control treatments 1 and 2 respectively. The effects of the treatments on the progression of the dental plaque, on the incidence of caries and on the oral flora in the rats are then studied.

A control group of rats follow treatment 1 based on soya bean protein. The results for this group serve as reference for treatments 3 and 4. A second control group of rats which follow treatment 2 (soya bean protein + 10% sucrose) serves as control for treatments 5 and 6.

	Treatments Diets	**1** (control)	3	4	**2** (control)	5	6
Number Of Rations	0.4 g $\mathbf{D_2}$ basic diet	**X18**	X18	X18	**X18**	X18	X18
	0.4 g $\mathbf{D_1}$ control with sugar				**X18**		
	0.4 g $\mathbf{D_0}$ control without sugar	**X18**					
	1 g of rennetted milk		X18				
	1 g of yogurt			X18			
	1 g of rennetted milk + 10% sugar					X18	
	1 g yogurt + 10% sugar						X18

Table 1 : Programme - Number of rations distributed for the treatments and controls 1 to 6.

Diets Ingredients	$\mathbf{D_2}$ "highly cariogenic"	$\mathbf{D_1}$ "slightly cariogenic"	$\mathbf{D_0}$ "non cariogenic"
Caster sugar	56%	10%	-
Skimmed milk "substitute" based on soya bean flour*	28%	28%	28%
Wheat flour	8%	54%	64%
Beer yeast	5%	5%	5%
Gevral protein	2%	2%	2%
Sodium chloride	1%	1%	1%

Table 2 : Compositions of the basic diet D_2 and of the controls D_1 and D_0 given according to Table 1.

*43.8% soya bean extract, 54.9% lactose, 0.8% $CaCl_2 \cdot 2H_2O$, 0.4% L-Met and 0.1% L-Lys.

The initial (T) and advanced (B) dental fissures, as well as the caries on the smooth surfaces (E) were analysed. The results are given in Table 3.

Treatments	TΔΔ	BΔΔ	EΔΔΔ
1 (control without sugar)	**11.5**	**9.2**	**17.5**
3 rennetted milk	10.3	6.1	5.4
4 yogurt	11.3	7.3	8.3
2 (control with sugar)	**11.8**	**9.9**	**16.9**
5 sweetened rennetted milk	11.9	10.3	10.9
6 sweetened yogurt	11.7	9.9	10.8

Table 3: Mean values per rat of the initial dental fissures (T), advanced dental fissures (B) and caries on the smooth surfaces (E).

ΔΔ = 12 fissures maximum, ΔΔΔ = 20 units of caries maximum.

The results show that among the 2 unsweetened fresh dairy products, only the rennetted milk corresponding to the invention causes a significant reduction in the T lesions (treatment 3 vs 1). Yogurt does not significantly affect the development of these lesions. When these products are sweetened (treatments 5, 6 vs 2), they have no effect on these same lesions.

The results of the tests also show that rennetted milk has a clear and significant action on the reduction of the B lesions, whereas the action of yogurt is statistically at the limit of what is significant (treatments 3, 4 vs 1). The sweetened dairy products (treatments 5, 6 vs 1) have no effect on these lesions.

As regards the action on caries on the smooth surfaces (E), the action of the 2 fresh dairy products is very significant, that of rennetted milk being substantially greater (treatment 3 vs 1). These same products when sweetened (treatments 5, 6 vs 2) have an effect which is still significant.

The unsweetened rennetted milk reduces all the types of caries more substantially than when it is sweetened (treatment 3 vs 5).

It has been possible to establish the following summary table (Table 4):

	Advanced dental fissures (B)	Caries on the smooth surfaces (E)
Control without sugar	9.2 caries = 100%	17.5 caries = 100%
Plain yogurt	21% fewer caries	53% fewer caries
Rennetted milk	34% fewer caries	69% fewer caries

Table 4 : Comparative percentages of reduction of caries (B) and (E).

The results show that these two fresh products have a clear anti-caries activity. However, the rennetted milk according to the invention which contains CGMP in a free hydrolysed form and calcified casein, shows a substantially higher anti-caries activity. This effect is even more pronounced against caries on the smooth surfaces.

Example 3

A composition for a fresh milk is prepared by mixing:

Rennetted milk prepared according to Example 1	10.0%
Demineralized whey powder containing 12.5% protein	64.9%
Milk fat	20.6%
Maize oil	4.4%
K_2HPO_4	0.1%

A composition for an infant formula is then obtained which has anticariogenic properties.

Example 4

An anticariogenic composition for baby food is prepared. For that, the following are mixed:

Rennetted milk prepared according to Example 1	12.6%
Demineralized whey powder containing 12.5% protein	22.9%
Lactose	34.9%
Milk fat	20.4%
Maize oil	4.6%
Minerals, micronutrients and water	4.6%

Example 5

A composition for a milk drink is prepared by mixing:

Rennetted milk prepared according to Example 1	10-15 g
Lactose	5-10 g
Water	70-90 g

The sum of these components should give 100 g.

Example 6

The rennetted milk prepared according to Example 1 is supplemented with 1 to 2% of variacin in bacteriocin powder form as described in patent EP 0,759,469.

The refrigerated composition obtained has the anticariogenic properties of rennetted milk and an extended storage life.

Example 7

A sweetened and flavoured refrigerated food composition is prepared from the rennetted milk obtained in Example 2. To do this, 5 g of xylitol and 2 g of flavouring agent are added to 100 g of rennetted milk obtained according to Example 2.

The composition obtained is a fresh dairy product having a nice sweet taste and an optimized anticariogenic activity.

Example 8

It is possible to prepare a sweetened and flavoured refrigerated food composition from the following ingredients:

Skimmed milk	78-82%
Cream containing 40% fat	10-12%
Skimmed milk powder	6-8%
Agar/gelatin	0.4-2%
Potassium sorbate	0.05-0.2%
Aspartame/acesulfame	0.02-0.06%
Flavourings/colourings (strawberry, banana and the like)	0.05-0.2%
Total	100%

The mixture is heated to 70°C and then homogenized at a pressure of 200 bar. It is pasteurized at 110°C for 6 min and then cooled to 43°C. It is rennetted with about 4 g of rennet 1/15000 per 100 kg of mixture. The product is packaged and then coagulated for about 1 hour 30 min and then cooled to 4°C.

It is possible to prepare a variant using, as source of sugar, about 7.5% of the following mixture: 1:4 to 4:1 of maltitol and 4:1 to 1:4 of fructose. In order not to exceed 100%, the quantity of skimmed milk is reduced.

The composition obtained is a fresh dairy product having a nice sweet taste and an optimized anticariogenic and anti-plaque activity. It contains a minimum of 0.05% CGMP in an active form and a minimum of 2% complexed casein.

Example 9

A stirred rennetted milk can be prepared from the mixture described in Example 8 but after cooling to 4°C, about 10% fromage frais or yogurt (so as not to exceed 100%, the quantity of skimmed milk is reduced) is added and then the mixture is stirred, made smooth and packaged. A stirred, flavoured fresh dairy product is obtained having an anti-caries and anti-plaque activity.

Example 10

A refrigerated food composition having an optimized anti-caries and anti-plaque activity can be prepared from the following ingredients:

Skimmed milk	30-35%
Cream containing 40% fat	27%
Skimmed milk powder	3-5%
Starch	2-4%
Maltitol/fructose	15-18%
Cocoa powder	2%
Fromage frais	10%

Trisodium citrate and flavourings are added and then the mixture is brought to 100% by adding water.

The milk-cream-milk powder mixture is heated at 110°C for about 2 minutes and then it is cooled to 43°C. It is rennetted with about 4 g of mixture for 1 h 30 min. It is heated to 85°C and then the other ingredients are added. The mixture is homogenized under pressure and treated at 130°C for about 40 seconds and packaged after cooling.

Example 11 : powdered anti-caries composition

Milk (skimmed where appropriate) is pasteurized and then cooled to between 10 and 40°C before adding rennet in an amount of about 0.1% and then allowed to act for 60 to 600 min. The mixture is homogenized under pressure and then the fats are added. The mixture is heated to 70°C, homogenized again and then heated to 120°C. It is concentrated by about 50% and the mixture is dried (spray-drying) after homogenization.

The powdered composition thus obtained has the same anti-caries and anti-plaque properties as the fresh dairy products according to the invention. The quantities of CGMP and casein in their active forms are as described above in the description.

Claims

1. Use of rennetted milk which contains both caseinoglycomacropeptide (CGMP) in hydrolysed form and coagulated casein, complexed with calcium, these two components being in their active form, to prepare food compositions intended for preventing or treating dental caries or dental plaque.

2. Use according to Claim 1, wherein the minimum quantity of CGMP present in the rennetted milk is 0.05% by total weight.

3. Use according to Claim 1 or 2, wherein the minimum quantity of complexed casein present in the rennetted milk is 2% of the total weight.

4. Use according to one of Claims 1 to 3, wherein the minimum effective quantity of rennetted milk is 10% by weight.

5. Use according to one of Claims 1 to 4, wherein the food composition is in powder form.

6. Anticariogenic food composition for preventing or treating dental caries or dental plaque, comprising rennetted milk which contains both caseinoglycomacropeptide (CGMP) in hydrolysed form and coagulated casein, complexed with calcium, these two components being in their active form.

7. Food composition according to Claim 6, wherein the minimum quantity of CGMP present in the rennetted milk is 0.05% by total weight.

8. Food composition according to Claim 6 or 7, wherein the minimum quantity of complexed casein present in the rennetted milk is 2% of the total weight.

9. Food composition according to one of Claims 6 to 8, wherein the CGMP is added in a manner to enhance the anti-caries and anti-plaque activity of the composition.

10. Food composition according to one of Claims 6 to 9, wherein the minimum effective quantity of the rennetted milk is 10% by weight.

11. Food composition according to one of Claims 6 to 10, provided in powder form.