

PATENT SPECIFICATION

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COMPLETE SPECIFICATION.

Improvements in or relating to Food Preparations Containing Nucleotides.

We, TAKEDA CHEMICAL INDUSTRIES LTD., of 27 Doshomachi 2-chome, Higashi-ku, Osaka, Japan, a Corporation organized under the laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

10 This invention relates to nutritional products, more particularly to milk foods.

15 Cow's milk is often employed as a substitute for human milk for feeding to infants. The cow's milk is used not only as fresh milk in liquid form but also as processed milk such as condensed milk, milk powder and fermented milk. However, cow's milk is not composed of the same ingredients as human milk. Therefore, untreated cow's milk is not a complete substitute for human milk.

20 Human milk is considered as a complete foodstuff comprising almost sufficient nutritious components, and therefore it has often been used as a standard for the measurement of nutritive value of foodstuffs. Human milk is regarded as a complete foodstuff, as infants usually grow remarkably well on it alone for several months.

30 Nowadays, women often do not like to

feed their infants with their own milk for various reasons, so that cow's milk is often used. As mentioned above, cow's milk is not a complete nutrient substitute for human milk, and so many products resembling human milk have been made therefrom. In preparing these products the most important problem is that of reducing to a minimum the differences between cow's milk in the products and human milk. Thus, differences between human milk and cow's milk in proteins, fats, lactose, mineral substances, vitamins and bifidus factor in respect of their quantity, quality and contents are minimized as far as possible. Therefore, enrichment of cow's milk with whey proteins, vitamin F and dactose has been investigated and practised in recent years.

On the other hand, in the field of biochemistry, attention has recently been drawn to nucleic acid as a fundamental substance in life. In the course of the present inventors' study, various interesting facts have been found. One such fact is that there is a characteristic difference in distribution of nucleic acid components between human milk and cow's milk. This fact was shown by the present inventors' comparative study on acid soluble fractions of both milks. An example of the study is shown in the following table:—

[Price 4s. 6d.]

TABLE

	Nucleotides*	human milk ($\mu\text{g}/100\text{ g.}$)	cow's milk ($\mu\text{g}/100\text{ g.}$)
5	Cytidine 5 ¹ -diphosphate choline (CDP-Choline)	trace	trace
	Cytidine 5 ¹ -monophosphate (CMP)	1080—1800	297
	Adenosine 5 ¹ -monophosphate (AMP)	90	—
	3 ¹ ,5 ¹ -Adenosine cyclic monophosphate (3 ¹ ,5 ¹ -AMP)	21—24	138
10	Guanosine 5 ¹ -monophosphate (GMP)	27—53	—
	Uridine 5 ¹ -monophosphate (UMP)	114—133	—
	Uridine 5 ¹ -diphosphate N-acetyl-lactosamine (UX1)	11	—
	Uridine 5 ¹ -diphosphate N-acetyl-lactosamine fucose (UX3)	160	—
15	Guanosine 5 ¹ -diphosphate mannose (GDPM)	67—91	—
	Uridine 5 ¹ -diphosphate N-acetyl glucosamine plus Uridine 5 ¹ -diphosphate N-acetylgalactosamine (UDP—AG+UDP—AGal)	260—361	—
20	Uridine 5 ¹ -diphosphate glucose plus Uridine 5 ¹ -diphosphate galactose (UDPG+UDP—Gal)	67—84	—
	Guanosine 5 ¹ -diphosphate (GDP)	34	—
25	Uridine 5 ¹ -diphosphate glucuronic acid plus Uridine 5 ¹ -diphosphate (UDP—GA+UDP)	127—218	—
	Orotic acid	—	6190

- Notes * (a) The nucleotides will be referred to hereinafter by the respective abbreviations shown in parenthesis.
 (b) In human milk, other kinds of nucleotide fractions can be found but they have not yet been identified.
 (c) μg means micrograms.

As can be seen from the above table, many types of nucleotides are present in human milk, while most of the nucleotide fractions found in cow's milk are present as orotic acid, which is regarded as a precursor of nucleic acid. It is interesting that there is such a remarkable difference between human milk and cow's milk in distribution of nucleic acid constituents, which are considered as a source of infant's nutrients.

Among these nucleotide fractions, UDP—AG, UX1 and UX3 are found in human milk. They are constructed from both UDP and sugars which are known as "bifidus factor" and are found particularly in human milk. Thus, "bifidus factor" is found in mother's milk in combined form with UDP. The "bifidus factor" causes a predominant occurrence of *Lactobacillus bifidus* in the intestinal tract of breast-fed infants, and therefore it is very important factor in protecting infants from bacterial intestinal diseases.

It is only recently that nucleotides have become easier to obtain as the result of improvements in microbiological or organic chemistry. Thus, 5¹-nucleotides may be produced industrially by the hydrolysis of

nucleic acid by an enzyme system of a micro-organism.

The present invention provides a nutritional product which comprises one or more soluble or dispersible carbohydrates together with nucleotides which are present in human milk but substantially absent in cow's milk (which nucleotides may be present in the free state or as salts), said product containing no active phosphatase.

The nutritional product provided by the invention possesses properties other than the known properties of nucleotides and cow's milk, so that babies can be reared on the product of the invention.

The carbohydrates can be present as, for example, cow's milk or processed products thereof (such as dried milk, evaporated milk or condensed milk), pregelatinised cereal powder, prepared baby food or fruit juice.

Thus we have found, for example, that cow's milk can be converted into a product resembling human milk and that nucleotides are very unstable in cow's milk owing to the presence of phosphatases (e.g. phosphomonoesterase and pyrophosphatase) which catalyse the hydrolysis of nucleotides into nucleosides and that the phosphatase can be

griseus (ATCC-10137), the mixture being allowed to stand at 40° C. for 15 hours. The mixture obtained is filtered by the aid of filter-aid (diatomaceous earth), and the filtrate is passed through a column packed with carboxylic acid resin (polymer bead resin, acrylic type, commercially sold under the Trade Name of "Amberlite IRC-50"). The eluant is concentrated under reduced pressure to obtain a nucleotides-containing concentrate.

The concentrate is admixed with the above-prepared cow's milk concentrate, then is spray-dried. To the prepared powder, β -lactose is added to make the whole weight 100 kilograms. The product may be canned under nitrogen gas.

EXAMPLE 6.

To 600 litres of raw cow's milk, there are added 10 kilograms of β -lactose and 10 kilograms of soluble polysaccharide (a mixture of dextrin and maltose) and the mixture is boiled.

To a small quantity of a pasteurized cow's milk are added 180 micrograms of disodium GMP, 550 micrograms of disodium AMP, 800 micrograms of disodium UMP, 300 micrograms of disodium GDP and 1.2 grams of UDP-GA. The solution is added to the 600 litres of milk treated as above, and then the mixture is condensed and spray dried. To the powdery product, the following quantity of vitamins and amino acid

	amount per 100 g. of product
Vitamin B ₁ ...	0.5 mg.
Vitamin B ₂ ...	0.9 mg.
Vitamin B ₁₂ ...	2 gamma
Nicotinic acid ...	4 mg.
Vitamin C ...	40 mg.
Vitamin A ...	2,000 International Units
Vitamin D ...	400 International Units
Cystine ...	200 mg.

and lactose were added to adjust the quantity of the product to 100 kilograms.

EXAMPLE 7.

To 100 kilograms of cow's milk powder which consists of 10 kg. of β -lactose, 10 kg. of sucrose, 6 kg. of dextrin, 3.5 kg. of maltose, 70.5 kg. of dried milk, 1.5 kg. of CaCO₃ and 48 grams of iron pyrophosphate there are added 9 grams of disodium CMP, 180 micrograms of disodium GMP, 550 micrograms of disodium AMP and 4 grams of disodium UMP. The mixture is well mixed and screened to prepare a phos-

phatase-free powdered milk. The screen used was a Tyler mesh 60 sieve.

EXAMPLE 8.

A mixture composed of 600 litres of raw cow's milk and 10 kilograms of sucrose is boiled and condensed.

Ribonucleic acid in yeast extract is hydrolyzed in the same manner as in Example 5, then AMP and GMP are separated by a conventional manner to leave a mother liquor containing CMP and UMP.

The mother liquor is added to the afore-prepared milk condensate, the mother liquor containing 8 grams each of CMP and UMP. To the mixture, there is further added 500 grams of disodium hydrogen phosphate, then it is spray-dried. To the product is added 20 kilograms of β -lactose to obtain 100 kilograms of powdered prepared milk.

EXAMPLE 9.

In a culture tank, *Streptomyces aureus* (ATCC-13404) is incubated at 28° C. for 2 to 3 days on a nutrient medium of pH 7.0 composed of soluble starch (4%), peptone (1%), meat extract (1%), dried yeast (0.2%), potassium dihydrogen phosphate (0.1%), sodium chloride (0.5%) (these percentages are in weight by volume percent). The culture filtrate is adjusted to pH 4.0, is then heated at 50° C. for 1 minute, then adjusted to pH 7.0 with sodium hydroxide. To 25 litres of this enzyme solution, there is added 10 kilograms of dried yeast plus 20 litres of water to suspend the yeast, then the pH of the suspension is adjusted to pH 8.0. The suspension is kept standing for 16 hours at 37° C., followed by removing the cells by centrifugation or filtration. The nucleotides-containing solution thus prepared is passed through a column packed with activated charcoal followed by elution with 1.5% aqueous ammonia to give fractions containing respectively UMP, CMP, GMP and AMP. Each of fractions is neutralized with sodium hydroxide solution and is concentrated to obtain the respective disodium salts.

A mixture composed of raw cow's milk (600 litres) sucrose (5 kg.), β -lactose (5 kg.), iron pyrophosphate (18 g.) and calcium carbonate (200 g.) is boiled, condensed under reduced pressure and spray-dried. To 20 kilograms of lactose, there are added previously prepared disodium CMP, disodium GMP, disodium UMP, disodium AMP, GDP and UDP-GA whose respective weights are 9 grams, 180 milligrams, 900 milligrams, 500 milligrams, 300 milligrams and 1.2 grams, then they are admixed homogeneously. The homogeneous mixture of 80 kilograms of the powdered milk so prepared and 20 kilograms of the mixture of

lactose and nucleotides forms the human milk-like powdered cow's milk.

EXAMPLE 10.

5 A ribonucleic acid-containing aqueous extract of *Torula* yeast is hydrolyzed by the process of Specification No. 912,790 employing a culture filtrate of *Streptomyces coelicolor* to (ATCC-13405) obtain a solution composed of a nucleotide mixture. The mixed nucleotide solution is concentrated and is neutralized with sodium hydroxide solution to make the nucleotide disodium salts followed by further concentration to dryness.

15 To 50 millilitres of a pasteurized cow's milk, there is added 2 to 3 grams of this disodium salt mixture. The milk solution of nucleotides mixture is added to 100 litres of cow's milk flowing into a flash pasteurizer. The processed milk flowing out of the pasteurizer may be bottled.

EXAMPLE 11.

25 A ribonucleic acid-containing aqueous extract of *Torula* yeast is hydrolyzed in a similar manner with a culture filtrate of *Streptomyces aureus* (ATCC-13404). The hydrolyzate is passed through a column packed with carboxylic acid resin (polymer bead resin, acrylic type, marketed under the trade name of "Amberlite IRC-50"), to remove components having toxicity and unpleasant taste or odour, is then neutralized with an aqueous solution of sodium hydroxide, and concentrated.

35 600 litres of raw cow's milk is enriched with 10 kilograms of β -lactose, 10 kilograms of sucrose and 9.5 kilograms of soluble polysaccharide (a mixture of dextrin and maltose), followed by boiling. To the processed cow's milk, there is added the prepared nucleotide solution which contains 10 to 15 grams of nucleotides. The mixture is concentrated to dryness, and an adequate amount of vitamins and amino acid as specified in Example 6 are preferably mixed in to prepare 100 kilograms of processed milk powder.

EXAMPLE 12.

50 A ribonucleic acid-containing aqueous extract of *Torula* yeast is hydrolyzed in a similar manner with a culture filtrate of *Bacillus brevis* (ATCC-8185). To 100 litres of milk which has already been boiled, there is added a concentrate of this hydrolyzate of yeast, in such a volume as to contain 2 to 3 grams of nucleotides. The mixture is heated to 40 to 55° C. under reduced pressure, homogenized, canned, then pasteurized to obtain 30 litres of condensed milk.

EXAMPLE 13.

60 A ribonucleic acid-containing aqueous ex-

tract of *Torula* yeast is hydrolyzed in similar manner by the aid of a culture filtrate of *Anixiella reticulispota* (ATCC-13828), followed by the treatment with ion-exchange resin to obtain a non-toxic nucleotides solution. To the solution, there is added 1½ times the volume of methanol and filtration is carried out to obtain a solution mainly containing UMP and CMP, and the solvent is distilled off.

The above prepared nucleotide mixture is added to one hundred litres of cow's milk which has been filtered and homogenized, and the mixture is spray-dried.

EXAMPLE 14.

A mixture of white rice powder and about 10 times its volume of water is kneaded, then is boiled for 30 minutes to gelatinize the starch in the rice, followed by filtration. To the filtrate are added the disodium salts of CMP, GMP, UMP, GDP and UDP—GA in the ratio of 27 grams, 540 milligrams, 2.7 grams, 900 milligrams and 3.6 grams relative to 100 kilograms of final product. The mixture is spray-dried to prepare an additive for milk products.

EXAMPLE 15.

Alkaline saline solution (pH 8.0) is used to extract 1 kilogram of yeast for 4 hours at 90° C., and is then centrifuged to remove solid matter. A culture filtrate of *Streptomyces griseus* (ATCC-10137) is added to the filtrate, and is kept standing at 40° C. for 15 hours to hydrolyze ribonucleic acid. The total hydrolyzate is passed through a tower packed with carboxylic acid resin (polystyrene bead resin, acrylic type, known under the trade name "Amberlite IRC-50"), then the eluant is concentrated to obtain a concentrated solution of various nucleotides.

A mixture of white rice powder and about 10 times its volume of water is kneaded, is then boiled for 30 minutes to gelatinize the starch in rice, and filtered. The whole volume of the nucleotides solution is mixed with the filtrate, the ratio between filtrate and nucleotide solution being the whole volume of the former to 10 kilograms of the final product to be prepared. The mixture is spray-dried to prepare a gelatinized cereal powder to be used for an additive for milk products.

EXAMPLE 16.

White rice powder is suspended into water so as to make the content of solid matter about 30%.

To the suspension, there is added a concentrated solution of various nucleotides prepared by the same manner as in Example 15, just before the suspension is conducted into a drum dryer. The mixing ratio is the

inactivated without destroying the total nutrients in cow's milk.

When milk passes into the stomach it coagulates to form a curd which is harder with cow's milk than with human milk and so is not as easy to digest as is human milk. We have found that when the carbohydrates in the product of the invention are present as cow's milk, the invention makes cow's milk easier to digest.

The taste or flavour of cow's milk containing the said nucleotides is similar to that of human milk, so that such treated milk can be drunk willingly by infants who dislike cow's milk. Furthermore, the quantity of cow's milk drunk by infants and the speed of drinking cow's milk by infants are both increased by the addition of the nucleotides to cow's milk. Thus, we have found that there is a close connection between the inclusion of these nucleotide fractions and the infants' liking of the taste. We have also found that the curd tension of cow's milk is lowered considerably due to the addition of the said nucleotides so that the digestibility of cow's milk is increased. The addition of the nucleotides to cow's milk should be effected just before or simultaneously with or after the inactivation of phosphatases in non-processed or processed cow's milk.

Nucleotides which are present in human milk but substantially absent in cow's milk are AMP, GMP, UMP, GDP, GDPM, UDPG, UDP-Gal., UDP-GA, UX1, UX3 and UDP-AG. In addition to these, CMP is present in cow's milk far less than in human milk. Therefore CMP is included as a nucleotide present in human milk but substantially absent in cow's milk.

These nucleotides can be classified into two groups, namely 5'-nucleotides themselves sugar esters of 5'-nucleotides.

The compounds of the first group are 5'-nucleotides, and can be prepared by hydrolysis of nucleic acid which can be extracted from living tissues, such as micro-organisms (e.g. yeast) and animal tissues. The hydrolysis may be effected by the action of an enzyme system produced by micro-organisms or an enzyme system of snake venom or of bovine small intestine. But, as the latter two enzyme systems cannot be used industrially, it is recommended to employ an enzyme system of a micro-organism. Enzyme systems which may be used include those of the micro-organisms *Streptomyces griseus*, *Streptomyces flavus*, *Streptomyces aureus*, *Streptomyces lavendulae*, *Fusarium roseum*, *Helminthosporium sigmoideum*, *Bacillus brevis*, *Bacillus subtilis*, *Anixella cingulate*, *Botriosphaeria ribis chromogena*, *Glomerella cingulata* and *Sordaria fimicola*. In other words, the enzyme system is produced from micro-organisms which may belong to the

order *Actinomycetes*, the class *Fungi Imperfecti* or to *Bacteria*. The enzyme system may be employed for the hydrolysis as the culture filtrate, cell suspension or extracted enzyme. (See for example, Applications Nos. 10016/59 and 23287/60 (Serial Nos. 912,790 and 933,826). Alternatively, hydrolysis may be conducted in such a manner that the micro-organism is incubated on a nutrient medium to which nucleic acid is added. In most cases, the enzyme system contains phosphatase, which would act on nucleotides to hydrolyse them into the corresponding nucleosides, so that a "phosphatase inhibitor" such as an arsenate, phosphate or cyanate is added to the reaction mixture (see Specifications Nos. 912,790 and 933,826). Alternatively the phosphatases may be inactivated with phenol.

The actual hydrolysis may be conducted in a conventional manner to cause reactions utilising enzyme system of micro-organisms. The hydrolysis usually is conducted at 20 to 30° C. for 2 to 5 days. The hydrolysate obtained contains various kinds of 5'-nucleotides, e.g. UMP, GMP, CMP, AMP and sometimes inosine 5'-monophosphate (IMP) which is converted from AMP through deamination by AMP-deaminase (which is also present in the enzyme system). UMP, GMP, AMP and CMP which is present in human milk but substantially absent in cow's milk can be isolated from the hydrolysate in a conventional manner, for example, the isolation may be conducted by column chromatography on, e.g., activated charcoal and ion exchange resins. The ion exchange resin may be a weakly acid or weakly basic one and GMP, UMP, CMP and AMP are collected easily. GDP and UDP can be synthesised by phosphorylation of the GMP and UMP obtained. Some of other nucleotide fractions, e.g. UDP-GA and GDPM, may be synthesised chemically starting from UMP and GMP respectively. In such a manner, most of the nucleotide fractions which are present in human milk but substantially absent in cow's milk are prepared.

Thus, a preparation comprising cow's milk and nucleotides and resembling human milk can be prepared by adding to cow's milk the nucleotide fractions which are present in human milk but substantially absent in cow's milk. Though the nucleotide fractions may be added as such, it is preferable to add nucleotides in the form of an alkali metal salt, e.g. sodium salt or potassium salt or salt with an amino acid such as histidine to avoid possible coagulation of cow's milk due to the acidity of nucleotides.

Cow's milk contains phosphatases capable of hydrolysing nucleotides into corresponding nucleotides. Therefore, phosphatases

should be inactivated or destroyed immediately after, simultaneously with or before the addition of the nucleotide fractions. Though the inactivation or destruction of phosphatases may be effected by the addition of a phosphatase-inhibitor (e.g. an arsenate, phosphate or cyanate), most inhibitors are unsuitable as additives for infants' food, and therefore the inactivation or destruction by phosphatase-inhibitors is avoided where possible. We have also found that phosphatases are sufficiently inactivated by simple heating for a short time, e.g. at 60° C. for 40 minutes, at 65° C. for 20 minutes, at 70° C. for 5 minutes, at 75° C. for 40 seconds or at 80° C. for only one second. Therefore, the inactivation may conveniently be effected by heating for a short time. Such a short heating does not affect the natural flavour or taste of cow's milk, nor is there any fear of the contamination of the product with unfavourable substances.

When cow's milk is marketed, it is generally necessary to pasteurize or sterilize it to avoid undesirable infections caused by such micro-organisms which may be present in the milk. The heating is usually effected at 65° C. for over 30 minutes or at 75° C. for over 15 minutes. Therefore, the addition of nucleotide fractions to cow's milk may be conducted just before, at the same time as or after the heating process, so that the phosphatase in cow's milk is completely inactivated or destroyed.

When cow's milk is processed into condensed milk or into powdered milk, it is generally passed through heating process in order to effect pasteurization or sterilization of milk and for lowering curd tension of milk. Therefore, the addition of nucleotide fractions may preferably be added just before or simultaneously with or after such a heating process. Addition may also be made to the final product. When fermented milk products such as yogurt and lactic acid-containing drinks are processed by the method of the present invention, the addition should be conducted on the final product having been pasteurized, because the fermented milk product generally contains micro-organisms which may produce phosphatase.

Briefly stated, the addition of nucleotide fractions being present in human milk but substantially absent in cow's milk should be effected in the absence of phosphatase or just before the phosphatase are inactivated.

GMP, UMP, CMP and other nucleotide fractions may be added to cow's milk so as to make their contents similar to that of human milk. That is to say, to 1 litre of unprocessed cow's milk, 10 to 20 milligrams (preferably 15 milligrams) of CMP, 0.2 to 0.4 milligrams (preferably 0.3 milligrams)

of GMP, 1.2 to 1.4 milligrams (preferably 1.3 milligrams) of UMP, 0.4 to 0.6 milligram (preferably 0.5 milligram) of GDP and about 1.0 to 3.0 milligrams (preferably 2.0 milligrams) of UDP—GA. Other nucleotide fractions may also be added, taking the difference between cow's milk and human milk in their nucleotides content into consideration. For example, 0.5 to 1.0 milligram of UDPG and 0.5 to 1.0 milligrams of UDP may be added to cow's milk.

The above mentioned ratio of nucleotide fractions to be added to cow's milk may of course be changed depending on the nucleotide content of cow's milk.

The addition may be carried out all at once to unprocessed cow's milk (i.e. still containing active phosphatase, which is removed subsequently) to be prepared, but there is a possibility that by doing this the product may not become a homogeneous mixture. If there is such a possibility, the nucleotide fractions to be added are first dissolved in a smaller amount of pasteurized cow's milk, then the mixture is further added to remaining cow's milk.

When it is required to add nucleotides to condensed or powdery milk, the nucleotides may be added in a concentration enough to be diluted, when it is drunk, to the nucleotide content similar to that of human milk. In such a case, the addition may be conducted before or simultaneously with or after concentrating or spray-drying the milk. The product may contain sugar, soluble carbohydrate, salts, vitamins, amino acids, and so on, to make the nutrients well balanced therein. In particular, the product may be enriched with sucrose, β -lactose, vitamin F, vitamin A, vitamin D, L-lysine and L-methionine. The enrichment may of course be conducted before or simultaneously with or after the addition of the nucleotides. If desired, the product may be homogenized in a conventional manner. Though the nucleotides to be added are those present in human milk but substantially absent in cow's milk, all such nucleotides need not be added if some of them are not readily available. For example, even if one or more of UDP, UDPG, UDP—AG, UDP—GA, UX1 and UX3 are not present, the infants' liking taste of the processed cow's milk of this invention is markedly improved in comparison with that of unprocessed cow's milk. In fact, the kinds of the nucleotides to be added to cow's milk are restricted in view of their availability. Therefore, the present inventors have investigated whether infants' liking of the taste of the processed cow's milk be effected when only some of easily available nucleotides are added to cow's milk, and found that the infants' liking for cow's milk can be improved by the addition of

only AMP, GMP, UMP and CMP, all of which are most easily available by the hydrolysis of nucleic acid.

The cow's milk processed as above may be further processed in known manner to secondary products, such as ice cream, only when the secondary process does not require the use of micro-organisms capable of producing phosphatases. And, if it is desired to add nucleotides present in human milk but substantially absent in cow's milk to fermented milk products, the nucleotides should be added just before or simultaneously with or after pasteurization of the product.

Another method for the addition of nucleotides present in human milk but substantially absent in cow's milk is to first prepare a composition containing such nucleotides and then to add so-prepared composition to unprocessed or processed cow's milk.

Suitable carbohydrates for this purpose include for example, α -lactose, β -lactose, mixtures thereof, sucrose, maltose, dextrin a pre-gelatinized cereal powder. Though raw cereal grains contain more or less phosphatase, it is inactivated by heating or boiling, as is known for preparing pre-gelatinized cereal powder. Therefore, the nucleotides can be added to such carbohydrate without any consideration of the phosphatase activity. But, if there is any fear that phosphatases are present in carbohydrate, the nucleotides may be added just before or simultaneously with or after complete inactivation of all phosphatases. The nucleotides are preferably added to such carbohydrates in a concentration such that the concentrations in the milk to be taken are as in human milk. Thus, the amount of CMP, GMP, UMP, GDP and UDP in 1 kilogram of the product may be from 0.2 to 0.3 gram, from 5 to 6 grams, from 0.02 to 0.03 gram, from 0.008 to 0.01 gram and from 0.03 to 0.04 gram, respectively. To unprocessed milk, 4 to 7 percent by weight of such nucleotides preferably is added. In addition the nucleotides may be mixed with salts, vitamins or amino acids to enhance the nutritional value of the cow's milk so prepared.

As described above, the infants' liking for cow's milk is sufficiently improved by the addition of only AMP, GMP, UMP and CMP, which are the most readily available nucleotides. These readily available nucleotides may be prepared in an easily utilizable composition. Thus, they may be prepared in such a form as powder or tablet, in which a constant quantity of these nucleotides is present to avoid such a trouble as weighing or computation before the addition. The nucleotides are not of course restricted to AMP, GMP, UMP and CMP, but may

include other nucleotides. The composition may also contain excipients or carriers such as lactose (α - or racemic), soluble starch and other soluble or dispersible carbohydrates so long as they have no toxicity.

Though the contents of respective nucleotides in the composition may be determined by taking the molar ratio of base components of nucleotides in human milk into consideration, the composition may preferably be prepared so as to make the ratio among AMP, GMP, UMP and CMP 1.5—2.0:1.0—1.5:6.5—7:20 by weight. The preparation may be dissolved in a final concentration of 25—30 milligrams per litre of cow's milk.

The product may, if desired, be used by dissolving it in a small portion of water or pasteurized cow's milk, followed by the addition of the solution to cow's milk to be drunk. The composition may contain minerals, vitamins or amino acids to enhance the nutrient value of so-prepared milk.

Another feature of the present invention is the addition of nucleotides present in human milk but substantially absent in cow's milk to so-called baby foods.

Examples of such baby foods to which the nucleotides may be added include strained, chopped and powdery foods prepared from egg-yolk, liver, chicken, animal meat, fish meat, vegetables, fruits and custard. In such materials to be prepared into baby foods, there are generally present phosphatases, but the preparation of baby foods requires heating or boiling. Therefore, the phosphatases are all inactivated in the preparation. The nucleotides should be added just before or simultaneously with or after the heating or boiling. To one hundred grams of baby food, there may be added CMP, GMP, UMP, GDP or UDP—GA in amounts of 10 to 20 milligrams, 0.2 to 0.4 milligrams, 1 to 2 milligrams, 0.4 to 0.6 milligram or 1 to 2 milligrams, respectively. The amount of baby food thus prepared which may be administered to a 4 to 6 months old infant is for example 10 to 30 grams per meal, or to a 7 to 12 months old infant 60 to 80 grams per meal.

Nucleotides may be employed in a pure state or in a crude state. For example, the hydrolyzate of nucleic acid may be used as such only after the toxic components and components having unpleasant taste or odour are removed. This removal may most simply be effected by allowing the hydrolyzate to pass over a column of ion-exchange resin or of activated charcoal. As the ion-exchange resin, there may be used weakly acid cation exchange resin or weakly basic anion exchange resin. The crude nucleotides mixture thus prepared contains various nucleotides other than those present in

human milk, but these do not affect the taste or flavour of the product nor the nutritive value. In addition, when the crude nucleotides mixture is employed, it is difficult to add each nucleotide in the desired quantity, but it may be possible at least to make the cow's milk, (to which is added crude nucleotides mixture) resemble human milk in its components. The quantity of such a crude nucleotide mixture to be added may be determined by reference to the analytical value of the mixture on the nucleotides contents therein.

Cow's milk contains 3',5'-AMP in rather larger quantities than does human milk, and orotic acid which is not contained in human milk. These nucleotides remain in the prepared milk of the present invention, but have no effect on nutritive value and no harmful effect on the infants. In order that the invention may be more fully understood, the following Examples are given, by way of illustration only. All percentages in these examples are in weight percent. Numbers attached to respective names of micro-organisms are accession numbers of the micro-organisms at American Type Culture Collection, Washington, D.C., U.S.A. In these examples, nucleotides are all used in the form of their disodium salts, but they may be replaced by other salts, e.g. potassium salt and ammonium salt, or by free nucleotides, if desired.

EXAMPLE 1.

To 600 litres of raw cow's milk are added 10 kilograms of β -lactose, 9.5 kilograms of soluble polysaccharide (a mixture of dextrin and maltose), 10 kilograms of sucrose and 1.5 kg. of calcium carbonate and 48 grams of iron pyrophosphate. The mixture is then boiled and reduced to about 200 litres volume. 9 grams of disodium CMP, 180 milligrams of disodium GMP, 900 milligrams of disodium UMP, 300 milligrams of disodium GDP and 1.2 grams of sodium salt of UDP-GA are dissolved in the boiled mixture. The mixture obtained is spray-dried and the adequate quantity of sucrose to make the whole weight 100 kilograms is added. The product may be canned with nitrogen gas in place of air, i.e. at the sealing of a can, it is placed under a reduced pressure (of about 30 centimeters water) and the pressure is increased with nitrogen gas of more than 90% purity up to ambient. Thereafter the can is sealed perfectly and, in this way, the oxygen retained in the can decreased to an extremely small amount.

EXAMPLE 2.

20 kilograms of β -lactose are homogeneously admixed with 9 grams of disodium CMP, 180 milligrams of disodium UMP,

300 milligrams of disodium GDP, 1.2 grams of sodium salt of UDP-GA. The mixture is added to 80 kilograms of a cow's milk powder to which sucrose, soluble polysaccharide (a mixture of dextrin and maltose), phosphorus, iron and calcium have been added in conventional manner. The cow's milk powder referred to in this Example was prepared in the following manner:—

1,500,000 International Units of Vitamin A and 400,000 International Units of Vitamin D were added to 450 litres of milk and the milk was then condensed. The condensed milk was spray-dried to give 70 kilograms of milk powder.

To the milk powder there were added 8 kilograms of sucrose, 2 kilograms of soluble polysaccharide (a mixture of maltose and dextrin), 48 grams of iron pyrophosphate and 1.5 kilograms of calcium carbonate and the mixture was thoroughly mixed to yield 80 kilograms of cow's milk powder. The mixture is mixed well to make the product homogeneous. The product may be canned with nitrogen gas in place of air.

EXAMPLE 3.

In 50 millilitres of pasteurized cow's milk, there are dissolved 1.4 grams of disodium CMP, 30 milligrams of disodium GMP, 130 milligrams of disodium UMP, 200 milligrams of sodium salt of UDP-GA. The solution is poured continuously into a stream of cow's milk flowing continuously into a pasteurizer at a constant rate, the ratio of the components being so adjusted that 50 millilitres of the solution prepared as above is mixed with each 100 litres of cow's milk. The pasteurization may be at 91° C. for 2 seconds. The product may be filled into bottles.

EXAMPLE 4.

To 100 litres of pasteurized cow's milk is aseptically added a solution of nucleotides whose composition is the same as that used in Example 3. The product may be filled into bottles.

EXAMPLE 5.

To 600 litres of raw cow's milk are added 10 kilograms of a mixture of 40% α -lactose and 60% β -lactose, 9.5 kilograms of soluble polysaccharide (a mixture of dextrin and maltose), and 10 kilograms of sucrose. The mixture is boiled and condensed under reduced pressure.

One kilogram of dried *Torula* yeast is extracted with 5% aqueous sodium chloride solution, (which was adjusted to pH 8 with NaOH), at 90° C. for 4 hours and the extract is centrifuged to remove solid matter. To 150 litres of the extract, there are added 50 litres of culture filtrate of *Streptomyces*

whole volume of nucleotide solution per 30 kilograms of the suspension. The suspension is gelatinized in the drum simultaneously with the drying process. This product may be used as an additive for milk products.

EXAMPLE 17.

Orange juice and apple juice are admixed with each other, and to the mixture there is added sugar. The mixture is agitated at 80° C. for 5 minutes. To 100 grams of the mixed juice are added 15 milligrams of disodium CMP, 300 micrograms of disodium GMP and 1.5 milligrams of disodium UMP. The mixture is then strained, canned and sterilized.

EXAMPLE 18.

Composition of tablets:—

Disodium AMP	1.72 milligrams per tablet
Disodium GMP	1.26 "
Disodium UMP	6.62 "
Disodium CMP	20.50 "
β -lactose	169.90 "
Total	200.00 milligrams per tablet

The above composition is pressed to form tablets in a conventional manner. One tablet is dissolved, before admixing with cow's milk, in 5 millilitres of warm water. The solution of the tablet is dissolved in 1 litre of phosphatase-free cow's milk or a solution of milk powder, to make a phosphatase-free cow's milk.

WHAT WE CLAIM IS:—

1. A nutritional product which comprises one or more soluble or dispersible carbohydrates together with nucleotides which are present in human milk but substantially absent in cow's milk (which nucleotides may be present in the free state or as salts), said product containing no active phosphatase.

2. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as cow's milk.

3. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as dried milk, evaporated milk or condensed milk.

4. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as pregelatinised cereal powder.

5. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as prepared baby-food.

6. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as fruit juice.

7. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides are more than one of the following namely, cytidine-5¹-mono-phosphate, guanosine-5¹-monophosphate, uridine-5¹-monophosphate, guanosine-5¹-diphosphate, uridine-5¹-diphosphate glucose, uridine 5¹-diphosphate galactose, uridine-5¹-diphosphate glucuronic acid and uridine 5¹-diphosphate N-acetylglucosamine.

8. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides comprise a yeast hydrolysate produced by the enzyme system of a micro-organism.

9. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides are present as their disodium salts.

10. A nutritional product as claimed in Claim 1, substantially as described with reference to any of the Examples.

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- whole volume of nucleotide solution per 30 kilograms of the suspension. The suspension is gelatinized in the drum simultaneously with the drying process. This product may be used as an additive for milk products.

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- Orange juice and apple juice are admixed with each other, and to the mixture there is added sugar. The mixture is agitated at 80° C. for 5 minutes. To 100 grams of the mixed juice are added 15 milligrams of disodium CMP, 300 micrograms of disodium GMP and 1.5 milligrams of disodium UMP. The mixture is then strained, canned and sterilized.

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	β -lactose	169.90 "

Total 200.00 milligrams per tablet

- The above composition is pressed to form tablets in a conventional manner. One tablet is dissolved, before admixing with cow's milk, in 5 millilitres of warm water. The solution of the tablet is dissolved in 1 litre of phosphatase-free cow's milk or a solution of milk powder, to make a phosphatase-free cow's milk.

WHAT WE CLAIM IS:—

1. A nutritional product which comprises one or more soluble or dispersible carbohydrates together with nucleotides which are present in human milk but substantially absent in cow's milk (which nucleotides may be present in the free state or as salts), said product containing no active phosphatase.

2. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as cow's milk.

3. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as dried milk, evaporated milk or condensed milk.

4. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as pregelatinised cereal powder.

5. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as prepared baby-food.

6. A nutritional product as claimed in Claim 1, wherein the soluble or dispersible carbohydrates are present as fruit juice.

7. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides are more than one of the following: namely, cytidine-5'-mono-phosphate, guanosine-5'-mono-phosphate, uridine-5'-mono-phosphate, guanosine-5'-diphosphate, uridine-5'-diphosphate glucose, uridine-5'-diphosphate galactose, uridine-5'-diphosphate glucuronic acid and uridine-5'-diphosphate N-acetylglucosamine.

8. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides comprise a yeast hydrolysate produced by the enzyme system of a micro-organism.

9. A nutritional product as claimed in any of the preceding claims, wherein the nucleotides are present as their disodium salts.

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