Abstract: A first aspect of the invention relates to a mineral-protein complex (Complex I), the complex including a mineral and a protein characterised in that the protein is derived from a milk source and wherein the milk source has a ratio of protein to calcium equal to or above 45:1, and wherein the mineral-protein complex includes over 1% w/w bound mineral. A second aspect of the invention relates to a mineral-protein complex (Complex II), the complex including an exogenously added mineral and a protein, wherein the mineral-protein complex is soluble in a solution at a physiological pH between 6.6 and 6.9 characterised in that the complex includes exogenous phosphorus. Also encompassed within the invention are methods of manufacture of the complex and resulting products, and use in the preparation of fortified products.

Published: with international search report (Art. 21(3))
Mineral Fortification Process and its Uses

TECHNICAL FIELD

The present invention relates to mineral-protein complexes and their uses as fortificants.

BACKGROUND ART

Essential metals (otherwise known as 'minerals' in nutrition science) iron, zinc, copper, manganese, magnesium, selenium, chromium are needed for many body functions, and are required by the body in sufficient quantities to meet its demands in order to maintain optimum health. These minerals are found in varying levels in different foods according to the source (i.e. magnesium from cereal products, iron and zinc from red animal muscle tissue, etc) and production location (i.e. high or low selenium soils) of that product. Economic, religious and ethical constraints, or simple personal food preferences, may result in certain populations or individuals consuming a diet that does not provide adequate levels of certain essential minerals for optimum health.

Fortification technologies provide opportunities to add an essential mineral(s) to products that would not usually be significant sources of the mineral(s). This means that a wider range of food products can contribute to the total dietary intake of the mineral(s), and thus provides consumers with alternative means of achieving the intakes required for optimum health. However, it can be technologically challenging to add minerals to foods, especially minerals that tend to readily interact with other food components, such as iron. This is particularly difficult in liquid food formats, where processing steps such as heating are involved. At present, fortifying foods or beverages with a physiologically-relevant level of bioavailable iron without the development of undesirable taste (metallic) and
appearance (colour changes which can occur either during processing or storage) is a significant challenge.

The natural forms of iron in the diet are haem and non-haem. Haem iron is a constituent of haemoglobin, the molecule that is responsible for carrying oxygen in the blood of most animals. For this reason, it is solely of animal origin, and is found in significant levels in meats such as beef, lamb and pork. It is highly bioavailable, due to its solubility in the alkaline conditions of the duodenum and jejunum (West and Oates, 2008), which allows it to be readily absorbed by the body. However, despite its high bioavailability, its animal origin presents difficulties for vegetarian and vegan populations.

Non-haem iron is naturally found in plant sources in either the ferrous or ferric form, and has a lower bioavailability due to low solubility at intestinal pH. The ferrous form of iron can be easily oxidised to its ferric state in the presence of oxygen, as is commonly encountered under processing conditions. Ferric salts of iron are precipitated as ferric hydroxide at pH >3, making them unavailable for absorption in the duodenum (Conrad and Umbreit, 2002).

The general dilemma in iron fortification of liquid and semi-solid foods (especially milk and dairy products) has been the issue of product stability. Traditional fortificants like ferrous sulphate or elemental iron are not suitable for the mass iron fortification of a range of food products due to lack of physico-chemical compatibility. Nutritional programmes involving iron fortification, that target young children and women, have attempted to fortify milk and dairy products due to their high nutritional value.

However, the reactivity of soluble (bioavailable) iron sources with constituents in liquid milk (caseins, fat and calcium in milk) has been shown to decrease the bioavailability of Fe both in vitro and in vivo studies in the past (Edmondson, 1971).
Reactivity of the iron sources also can translate into unpalatable products which is a further disadvantage. This reason has been the main deterrent in using milk as a vehicle for iron fortification.

The general consensus is that greater bioavailability is found in iron ingredients which have increased solubility at the duodenal pH (6.6-6.9). Compounds like ferric pyrophosphate, which are poorly soluble, have been used for fortification of dried milk and dairy products. However, its reported bioavailability is highly variable (Hurrell, 2002).

Chelated forms of iron have emerged as a convenient choice, as they are soluble at a physiological pH and are therefore available for absorption within the body. As the iron is bound to a ligand, it is prevented from interacting with other compounds present in the food matrix. However, despite their benefits from a functional and bioavailability perspective, chelates such as sodium ferredetate and ferrous bisglycinate are not presently used as a mass fortificant because of their reactivity at high temperatures (especially in the presence of polyphenols), as well as a high cost of raw materials.

An alternative that has been explored is to chelate iron with protein, such as casein, which is naturally present in milk. However, earlier commercial and research applications of binding iron to milk proteins (e.g. WO 2000/51446) have not been successful because of the formation of insoluble precipitates at higher levels of iron addition (>8mM). The levels of iron loading in this earlier patent was therefore unable to exceed 1% of the dried powder, which represents a ratio of casein to iron in the powder of approximately 92:1 assuming the protein content to be 92% of the said powder. Such products cannot be applied to beverages like milk, fruit juices etc because they could generate haze when added to transparent beverages and solutions.
In another example Raouche and co-workers added 20 mM iron (3.62% of milk protein Dry Matter (DM) basis) to milk at chilling temperatures, wherein the iron was bound to the caseins in micellar form. More than 90% of the added iron was bound to the caseins in the colloidal phase of milk. However, when milk with added iron was heated at 90°C for 10 min precipitates were observed (Raouche et al., 2009).

All references, including any patents or patent applications cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents form part of the common general knowledge in the art, in New Zealand or in any other country.

Throughout this specification, the word "comprise", or variations thereof such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

**DISCLOSURE OF THE INVENTION**

In the broadest aspect, the present invention relates to the provision of improved mineral protein complexes. Advantages of the complexes formed may include an
improved solubility and heat stability, ease and lowered costs in their manufacturing, and wider applications in food, beverage and therapeutic products.

The advantages of the mineral protein complexes, methods of manufacture and uses described herein will become more apparent with the ensuing description. Two embodiments of the present invention are outlined below under the Headings "Complex I" and "Complex II". It should be appreciated that the preferred embodiments of each complex may be utilised by the other complex, and vice versa.

In the following disclosure the contents of calcium, iron and phosphorus have been described as ratios with respect to the protein content in the samples. To further clarify; in the case of normal cow milk there is a concentration of 32g protein, 1000mg phosphorus and 1200mg of calcium per litre of milk, which will achieve a ratio of 26:1 (protein:calcium) and 32:1 (protein: phosphorus) respectively. A reduction in the calcium content will increase this ratio (protein/calcium), while an increase in the phosphorus will decrease this ratio (protein:phosphorus). Milk contains negligible amounts of iron and the external addition of iron is represented in terms of protein:iron ratio. Understandably, an increase in the concentration of iron will decrease the ratio of protein:iron.

Wherever low calcium is said it means a protein/calcium ratio in milk or sources of milk protein with protein/calcium ratio greater than 58:1 or a casein/calcium ratio greater than 45:1 (cow milk contains 32 g protein in which casein constitutes 25 g in 1 litre of milk).

Complex I

According to one aspect of the present invention there is provided a mineral-protein complex, the complex including
a) a mineral component; and

b) a protein

characterised in that the protein is derived from a milk source and wherein the milk source has a ratio of protein to calcium equal to or above 45:1 and wherein the mineral-protein complex includes over 1% w/w bound mineral.

According to a further aspect of the present invention there is provided a method of manufacturing a mineral-protein complex as discussed above, the method characterised by the step of:

a. adding a mineral to a milk source with a ratio of protein to calcium equal to or above 45:1 such that the mineral-protein complex includes over 1% of bound mineral.

Advantages of Complex 1

The inventors found that by using a milk source with a low level of calcium for example, as outlined in Figure 1, an improved fortification complex may be obtained compared to the prior art, particularly with regards to heat stability of the complex and the ability to load equal or higher amounts of the mineral whilst retaining stability and bioavailability.

It should be emphasised that the present invention may utilise a milk source with a low level of calcium which has been already provided, or may result from processing to remove calcium from a milk source. There are well-known techniques available to remove calcium from a milk source, such as ion exchange process, membrane processes, their combination and the like.

Without wishing to be bound by theory, the inventors consider the significant
advantages of the present invention are arising because the calcium, which normally binds with high affinity to the milk proteins, is being removed thus opening up binding sites for a mineral(s) to bind to the milk proteins. Therefore, it is possible that the ability to bind higher amounts of mineral(s) without certain disadvantages such as precipitation, for example, may be possible.

To provide an example using the method as described herein, the inventors were able to achieve an optimum ratio of protein/iron of 19.5:1 (equating to about 5.1% w/w loading of iron to protein in complex 1), although higher levels of iron loading were also able to be achieved. Additionally, the complex was found to be stable in a soluble form at these ratios and mineral loading, and it is considered this stability and higher loadings will be beneficial for inclusion in food and beverage products.

This is a significant improvement over loading of iron and stability as reported in WO 2000/5144, namely only 1% w/w iron loading in final powder (expected protein/iron ratio 92:1). In the current process, the inventors were able to achieve an optimum protein/iron 19.5:1 and still provide a very stable product, unlike as reported in Raouche et al, 2009. This improvement in the higher loading of the mineral and stability of the complex is thought to be attributed to the reduced calcium levels in the complex.

The invention may help to overcome problems associated with fortification of milk products including precipitation of protein, decreased stability particularly at the high temperatures experienced with processing of liquid and semi-solid food products and limitations to the amount of mineral that can be added during the fortification process. Furthermore, the preparation of these soluble mixes may enable iron fortification to liquid beverages, without affecting the shelf stability of liquid beverages.

Additionally, the present method results in a complex which is soluble at
physiological pH, unlike many of the prior art documents. In the past when ferric iron has been bound to casein through a precipitation process, it has been found that the bioavailability of the iron from such complexes is similar to that of ferrous sulphate (Zhang and Mahoney, 1989, Kim et al., 1995), which is considered to have very good bioavailability for a non-haem iron source. Given the nature of the present invention is similar in terms of the binding of a mineral to casein, it is expected to demonstrate bioavailability of a similar level.

A further advantage of this method is that it may use inherently available proteins in milk (such as casein). This may help to reduce manufacturing time and resources needed and so forth.

A further advantage of this process (and its resulting complex) is that the methods which may be used to remove calcium have no substantial effect on other constituents in the milk, which are left substantially unchanged. Again, this helps to keep the end product closer to the original milk composition.

**Preferred embodiments of Complex I**

Throughout this specification the term milk source should be taken as meaning whole milk or a component thereof sourced from a lactating animal.

Preferably, the lactating animal is a mammal. This is because, as will be outlined further below, all mammals have casein (a particularly preferred protein) in their milk.

Preferably, the milk source is from cow's milk. Alternatively, the milk source could be from human, sheep, buffalo, goat or another mammal that has relatively high levels of casein in the milk source or mixtures thereof. For example, casein makes up approximately 80% of proteins in cow's milk and buffalo milk, and about 20-50% of the proteins in human milk.
Throughout this specification the term protein should be taken as meaning any polypeptide molecule that has been either synthetically or naturally derived.

Throughout this specification, the phrase "low level of calcium" should be taken as meaning a protein/calcium ratio greater than that in normal milk. Normal milk has approximately 1200 mg of calcium constituting a protein/calcium ratio of 26.6:1.

Therefore, the protein/calcium ratio in the milk source is equal to or above 45:1, and this clearly constitutes a low level of calcium compared to what is present in normal milk.

Preferably, the protein/calcium ratio in the milk source is greater than 58:1. More preferably, the protein/calcium ratio in the milk source is approximately between 58:1 and 640:1.

In this embodiment the protein/calcium ratio is, preferably between 70:1 to 95:1, as an example 83:1.

This represents a significant decrease (approximately a decrease of 70%) of calcium present in the milk compared to milk which has not had calcium removed.

More preferably, at least 50% of the calcium is removed from the milk source.

Most preferably, approximately 70% of the calcium (w/v) has been removed from the milk source.

The inventors identified that removing 70% of the calcium from milk may be sufficient to solubilise more than 95% of the colloidal milk proteins (e.g. casein micelles). This improved the heat stability and physico-chemical properties of the milk proteins favouring the soluble complex formation.

Throughout this specification, the term mineral should be considered any mineral
which may be of physiological benefit to an animal (such as a human) and may be
delivered to an animal via the fortified mineral-protein complex. For example,
typical minerals of physiological value considered most applicable to the present
invention include those such as calcium, sodium, potassium, iron, zinc, copper,
manganese, selenium or chromium.

In the context of the present invention (for both complex I and II), it should be
understood that the fortification process relies on addition of at least one
exogenous mineral. In agreement with the general understanding in the industry,
and within the context of the present invention, the term exogenous should be
understood to mean that the mineral is externally added and is not provided
endogenously by the protein. It should also be understood that within the mineral-
protein complex, an amount of endogenous mineral may also be present. To
provide an example, endogenous calcium may still be bound to the casein. Yet in
addition, the protein complex may be fortified with exogenously added iron.

Preferably, the mineral is iron.

The preference to fortify the complex with iron comes back to the clear need to
provide soluble inexpensive fortified iron complexes and to address the problems
as outlined previously. However, the inventors acknowledge that the present
concept may be used to fortify a complex with other minerals beyond iron, such as
zinc, copper, manganese, selenium or chromium. Potentially inadequate intakes
of these minerals in animals present opportunities to utilise the present invention in
a similar mechanism. One skilled in the art would appreciate other minerals may
be substituted for iron and also bind to many proteins to form a complex.

Preferably, the iron is ferric and/or ferrous salts of iron. For example, ferric chloride
may be used. Alternative ferric iron sources such as ferric sulphate pentahydrate,
ferric phosphate, ferric pyrophosphate, etc. may be used without departing from the
scope of the invention. Ferric iron will bind more efficiently to caseins than ferrous iron owing to the binding characteristics of their respective iron oxidation states.

However, it should not be ruled out that ferrous iron may be used in the present invention.

Preferably, the protein from the milk source is selected from caseins, whey proteins and their individual fractions or mixtures of the same.

More preferably, the protein is casein.

A protein of particular interest is casein, which is inherently present in milk. In US 2003/0206939, it is outlined how various micronutrient components (e.g. minerals, enzymes, vitamins) which have affinity to casein proteins as a result of positive and negative groups along the length of the casein polypeptide chain.

Although casein represents a particularly preferred protein to be used in the present invention (either naturally or synthetically derived), it should be understood that many other proteins from a milk source may be used with Complex I.

It is known that casein binds calcium from milk to form colloidal casein micelles.

The inventors have identified that an advantage of removing calcium from milk is it may help to break down the casein micelle structure and thus allow solubilisation of individual caseins which become available to bind to the mineral (e.g. iron) once added.

There are many well-known techniques available to remove calcium from a milk source. On the other hand, many have tried to fortify milk with micronutrient components such as iron (GAUCHERON, F. 2000. Iron fortification in dairy industry. Trends in Food Science & Technology, 11, 403-409.). However, until now
it has not been thought to actually combine these two principles to arrive at a significantly improved complex.

The mineral-protein complex includes above 1% w/w bound mineral.

More preferably, the mineral-protein complex includes between 1% to 20% w/w bound mineral.

Even more preferably, the mineral-protein complex includes between 4 to 8% w/w bound mineral.

For both Complex I and II (discussed further below), the resulting complex was found to be very stable and soluble, and most likely will portray significantly improved functionality when incorporated into food and beverage products than the prior art complexes. Therefore, providing fortified complexes with higher loading of minerals such as iron (even at 1% w/w) represents a significant improvement over the prior art.

Also, these embodiments regarding % w/w of mineral bound reflect that although amounts lower than 1% w/w may be beneficial in some circumstances, a higher concentration of bound mineral may be much more commercially and physiologically useful.

There is a balance to be optimised with higher mineral fortification of the complex and ensuring stability of the complex. Indeed, the inventors have exemplified binding of 7% w/w loading (see Examples 3-5 with loading of 25 mM iron) while still ensuring the complex remains stable. It is quite possible that concentrations of up to 20% w/w mineral bound to the complex may be achieved. It should be understood that, depending on the application of the mineral-protein complex, different amounts of mineral bound to the complex may be developed for use. The inventors foresee that a 4% w/w loading of mineral is most applicable towards
various commercial uses, such as in milk powder fortified with iron.

Preferably, the mineral-protein complex includes additional phosphorus. The normal ratio of protein to phosphorus in milk is 32:1.

Preferably, the protein complex includes an amount of phosphorus which may decrease this ratio of protein:phosphorus to 8:1, or 6.25:1 for casein:phosphorus.

Below the above ratio, the inventors believe precipitation of proteins along with iron and phosphorus may occur.

A discussion of the advantages of adding phosphorus (and proposed mode of action) is outlined further in the next section. Any phosphorus containing food grade compound may be used with the present invention. However, one such example is K$_2$HPO$_4$.

Preferably, the complex of the present invention is used as a food additive or ingredient within a nutritional beverage product, food product, therapeutic/pharmaceutical composition or animal feed composition.

*Preferred method of manufacture of complex I*

A particularly preferred method of manufacture of Complex I is shown schematically in Figure 1.

Preferably, the milk source is a milk in liquid form inclusive of whole milk, skimmed milk, low lactose milk, ultrafiltration retentate concentrated milk and or mixtures thereof.

Alternatively, the milk source is one from powder form.

Types of milk powder which may be used include milk protein concentrate powder (MPC), calcium depleted MPC powder, whole milk powder, skim milk powder
(SMP) (or lactose reduced SMP), or phosphocaseinate powder. The protein concentration of the resulting milk source solution may vary from 1-12.5%.

Preferably, the milk is stirred, or in case of powder source is then dissolved, in an amount of water and mixed at a temperature between 2-95°C. Most preferably, the temperature is between 2-10°C, for reasons which will become apparent later.

The mixing step may last about 30 minutes.

After mixing, calcium may be removed if necessary to provide the low level of calcium as required.

One may start with a low-level calcium milk source, or may prepare such from a milk source initially with normal levels of calcium, as discussed further below.

Preferably, the method includes removing the calcium from the milk source using ion exchange.

In the ion exchange process, Na⁺, K⁺ or H⁺ form of resin may be used individually or in a mixed form. A strong acidic cation exchange resin, or a mixture of strong and weak forms may be used. Most preferably, the resin is a weakly acidic cation exchange resin of K⁺ form.

An advantage of ion exchange is that removal of calcium by this process may help to result in minimum alteration in the quantities of minerals present in milk other than calcium. This may play an important role in the creation of soluble complexes.

Preferably, the amount of resin used is 0.1-80% w/v.

More preferably, the amount of resin used is 0.1 to 50% w/v.

Preferably, the method includes reducing the amount of calcium in the milk source to a protein/calcium ratio to be greater than 58:1.
More preferably, the method includes reducing the amount of calcium in the milk source to a protein to Ca ratio of approximately 106:1.

Preferably, the method includes removing the amount of calcium in the milk source by at least 50% w/w and up to 100% w/w of the calcium from the milk source. Most preferably, the method includes removing the amount of calcium in the milk source to about 70% of the initial quantity.

Calcium removal may be monitored through a number of ways. One such example is a titration method using Patton Reeder reagent (Patton and Reeder, 1956).

To stop the ion exchange process, ion exchange resins in contact with milk may be removed via clarification. A wide variety of steps may be used to stop the ion exchange process, including centrifugation and filtration. Other methods may be used without departing from the scope of the present invention.

Preferably, the pH is maintained between 6.0 to 8.5 using at least one pH regulator.

More preferably, the pH is maintained at approximately 6.5 to 7.5.

The pH may need to be adjusted between 6.5 and 7.5 after ion exchange due to the change in calcium levels. To increase and decrease the pH, a pH regulator such as sodium hydroxide or hydrochloric acid or like, respectively, may be used.

Additionally certain minerals which may be added, such as ferric chloride, are highly acidic. These may precipitate the proteins if they are added to the milk protein. Therefore, maintaining this preferred pH will therefore help to prevent precipitation of proteins from the solution.

Unlike the prior art, the present invention allows inexpensive ferric compounds to be used and be soluble at a pH well above 3. Therefore, the present invention provides a soluble iron-protein complex which may be advantageously retained at a
physiological pH (6.5-7.5) which renders the complex to be available for absorption within the body. The process could also be performed anywhere between pH 8.5 and 6.3 with similar results.

Preferably, the calcium is removed whilst retaining the temperature between approximately 2-10°C.

Maintaining the temperature within this range has a number of advantages. It helps to prevent bacterial growth, and helps to control the rate of ion exchange. Also, β-casein, a major casein in milk, exists as a monomer at these temperatures. Therefore, this temperature may help to release the calcium from the micelles during the ion exchange process. Calcium phosphate is more soluble at lower temperature which may aid in ion exchange.

In the case of adding minerals such as iron, removing lactose may allow increasing the concentration of mineral in the complex.

A decrease in the ratio of protein:iron may be achieved by addition of phosphorus source such that the above ratio decreased from 28:1 (without phosphorus addition) to 19.5:1 (with phosphorus addition of 1000 mg/litre of milk), with further improvements expected.

As discussed previously, a protein:iron ratio of 28:1 equates to approximately 5.1% w/w binding of iron to the protein.

Preferably, the method includes an optional step of phosphorus addition to the low calcium milk source. In one embodiment, the phosphorus containing compound is an orthophosphate like $\text{K}_2\text{HPO}_4$. However alternative compounds are clearly envisaged, as discussed further in this specification (Complex II).

Preferably, additional phosphorus is added to the milk source solution.
More preferably, phosphorus is added to the milk source to provide a protein:phosphorus ratio between 64:1 to 8:1.

The inventor found this level of phosphorus was beneficial to improve increased iron (or other mineral) loading and complex solubilisation.

Most preferably this protein:phosphorus ratio is approximately between 32:1 to 8:1.

In the embodiment where iron-protein complexes are to be formed, the method includes slowly adding an iron containing compound to the calcium depleted milk source. One such iron containing compound is FeCl$_3$-6H$_2$O. A solution such as 0.01 to 0.5 M FeCl$_3$-6H$_2$O may be used for this process.

Minerals, such as ferric chloride, which may be added to form the fortified complex may be highly acidic. Subsequently, alterations in the milk pH may precipitate the proteins if they are added to the milk source. Therefore, maintaining a preferred pH between 5.8 to 10.5 (preferably 6.5 to 7.5) may help to prevent precipitation of proteins from the solution.

Preferably, the temperature is maintained between 2-8°C when the mineral (e.g. iron) is added. This again helps to maintain the protein (e.g. casein) as monomers to promote complex formation with the mineral.

Once the mineral is added, the resulting solution may be mixed for a period of time such as 30 minutes at between 2-8°C. This mixing may help to promote complex formation.

The solution may be clarified to remove precipitated or unwanted matter. The solution may be formulated into a powder by concentration and any suitable drying process, such as spray drying. Powder forms of the complex are considered to be particularly useful to increase shelf life compared to keeping the complex stored as
a solution. Furthermore, powders may be more easily handled and are versatile when used for the addition to food/beverage and/or pharmaceutical purposes.

It should be appreciated that the complex may instead be kept as a solution until further use.

**Complex II**

According to a further aspect of the present invention there is provided a mineral-protein complex including an exogenously added mineral and a protein, wherein the mineral-protein complex is soluble in a solution at a physiological pH between 6.6 and 6.9 characterised in that the complex includes exogenous phosphorus.

According to a further aspect of the present invention there is provided a method of manufacturing a mineral-protein complex as discussed above, wherein the method is characterised by the steps of

a. adding exogenous phosphorus to a protein; and

b. adding an exogenous mineral to the protein to form the complex.

**Summary of advantages of Complex II**

After developing the invention and advantages of Complex I, the inventors then devised an alternative embodiment as provided in Complex II which provided a range of different advantages and very positive results.

Similar to the advantages of Complex I, the method of preparing Complex II is relatively easy and cost effective compared to prior art techniques. However, as there is no need to remove calcium from milk, the present method may present an
even simpler process than that of Complex I. This is primarily because the process
may utilise proteins such as sodium caseinate which are purchased or otherwise
provided for in a pre-purified state.

Compared to the prior art, Complex II is a significantly improved mineral fortification
complex as it is again highly soluble and is not prone to precipitation or
aggregation. These are important advantages as they allow for easier storage and
use for various commercial products. Similar to Complex I, Complex II is stable at
physiological pH, unlike many of the prior art complexes. Furthermore, a higher
concentration of mineral bound to casein may be achieved in the final powder e.g.
final ingredient could contain 8% by wt of iron, and again these preliminary results
are expected to be improved upon. This is a major improvement to prior art
complexes which report loading of only 1% w/w iron in powder form (protein/iron
92:1).

A higher concentration of mineral such as iron allows the complex to be used for a
wide variety of uses. For instance, it may allow a greater dose of iron in a lower
volume/mass of a food product.

Many other advantages of these complexes are listed and discussed within this
specification.

Complex II relies on the addition of exogenous phosphorus to the protein to be
used for forming the complex. For simplicity, we again refer primarily to casein as
the protein. However, it should be understood that other proteins may be used with
the present invention without departing from the scope thereof.

The inventors identified that the added phosphorus plays an important role in the
formation of these stable, soluble complexes. Without wishing to be bound by
theory, it is thought that phosphorus may act by increasing the surface charge on
the complex thereby preventing the aggregation and consequent precipitation of the protein.

Caseins are known to be mineral chelators, which bind minerals such as iron mainly through the coordination complexes formed between the mineral and oxygen of the clusters of phosphoserine residues available throughout the structure of caseins. However, binding of iron to these caseins results in a decrease in the surface charge, thereby causing aggregation and precipitation of proteins. It may be possible that phosphorus acts by increasing these surface charges through mechanisms still unknown thereby preventing the aggregation of proteins.

Furthermore, the preferred process of making both complex I and II is conducted at temperatures of about 210°C where proteins such as casein exist partly as monomers due to absence of hydrophobic interactions at such temperatures. The existence of casein in the monomeric form might further provide binding sites for minerals such as iron thereby increasing the amount of minerals that could be bound to caseins.

These results could not have been logically predicted. This is because in past studies when phosphorus and calcium have been added to sodium caseinate, it had caused precipitation of the protein. Therefore, one skilled in the art would have assumed that upon on addition of iron in place of calcium, substantial precipitation and/or loss of stability would also have occurred.

*Preferred features of complex II*

Preferably, the protein is a phosphoprotein.

Preferably, the phosphoprotein is casein.

However, the inventors acknowledge that other proteins such as egg phophovitin
have similarities to casein which suggest a comparable level of binding and stabilization would occur following phosphorylation of the protein.

Other proteins such as soy protein, cereal protein and algal protein may also be used, albeit potentially with varied levels of phosphorylation and/or binding to produce a soluble and stable iron protein complex.

Preferably, the casein containing compound is sodium caseinate, potassium caseinate, ammonium caseinate, lactic casein and/or derivatives or fractions of caseins.

Preferably, the mineral is iron. Preferably, the iron is ferric iron. For example, ferric chloride may be used. Alternative ferric iron salts such as ferric sulphate pentahydrate, may be used without departing from the scope of the invention.

Similarly, a ferrous iron source may be used. The preference to fortify the complex with iron comes back to the clear need to provide soluble inexpensive fortified iron complexes.

However, the inventors acknowledge that the present invention may be used to fortify a complex with other minerals beyond iron, such as zinc, manganese, selenium or chromium. Requirements for all these minerals in animals present opportunities to utilise the present invention in a similar mechanism. One skilled in the art would appreciate other minerals, or mixtures of minerals, may be substituted for iron.

Preferably, the mineral-protein complex includes above 1% w/w mineral bound to protein.

More preferably, the mineral-protein complex includes between 1% to 20% w/w mineral bound to protein.
Even more preferably, the mineral-protein complex includes between 1 to 9% w/w mineral bound to protein.

The advantages of these loadings of mineral have been previously discussed in relation to Complex I, and the same reasoning applies for Complex II.

It should be appreciated that in the case of casein for example, \( \alpha_1 \), \( \alpha_2 \) and \( \beta \) caseins are highly phosphorylated, whereas other variants of casein such as \( \kappa \)-casein are sparsely phosphorylated. The phosphorylation patterns of casein subtypes are well documented, for example as outlined on page 1 of US 2003/0206939, which is herein incorporated by reference. This is also the case for many other proteins which may be used according to the present invention.

*Preferred Method of Manufacture of Complex II*

A particularly preferred method of manufacture of Complex II is shown schematically in Figure 2.

The method is discussed more generally below.

Preferably, the protein used is a casein containing compound.

Preferably, the casein containing compound used in the method is sodium caseinate, potassium caseinate, ammonium caseinate, lactic casein and/or derivatives and fractions of caseins. Such compounds may be readily obtainable from suppliers in a pre-purified state. As discussed previously, this avoids the need for processing or purification steps as used in the preparation of Complex I.

Preferably, the method includes dissolving the protein in water to form a solution. This dissolving step may be performed at a relatively higher temperature such as between 40-60°C to aid in the dissolving process. Once dissolved, the solution may preferably be chilled to a lower temperature, preferably between 2-10°C for reasons
discussed previously. However, the process may be performed at temperatures between 2-95°C.

Preferably, the protein concentration in the solution is configured to be between 1-12.5% w/v. Most preferably, the protein concentration in the solution is configured to be between 1-5% w/v.

After the protein solution is chilled, this is a convenient point at which phosphorus may then be added to the protein solution.

Most preferably, the phosphorus is added to the protein solution prior to the addition of the mineral. This may help to prime the protein solution for effective binding of the mineral.

The source of phosphorus may be food grade orthophosphate or polyphosphate or linear phosphate salt, as mono, di, trisodium, potassium, ammonium, magnesium or calcium phosphates, as well as phosphoric acid and/or mixtures thereof.

Preferably the source of phosphorus is K$_2$HPO$_4$. The normal ratio of casein to phosphorus is 65:1. A lower ratio than this is required to achieve the aforementioned benefits.

Preferably, an amount of phosphorus is added to the protein solution such that the ratio of protein to phosphorus is between 5:1 to 30:1. Most preferably, the ratio of protein (e.g. casein) to phosphorus is between 12:1 to 22:1.

Preferably, the mineral is added to the protein solution after the addition of phosphorus.

Preferably, the mineral is iron. However, it has already been emphasised that many other types of minerals may be used in a similar manner to bind to proteins such as casein.
Preferably the iron is ferric iron. One such source of ferric iron is FeCl₃, although others are clearly envisioned.

Preferably the ratio of protein to iron (e.g. casein) is between 200:1 to 2:1.

Most preferably, the ratio of protein to iron (e.g. casein) is between 100:1 to 10:1.

As previously discussed, as ferric iron is acidic, it may be appropriate to again adjust pH to within the preferred range using suitable pH regulator(s).

Preferably, the resulting protein solution is mixed for a period of time at 5-10°C. This may help to allow time for the mineral to bind to the protein to form the complex. Again, the preferred temperature is thought to help this binding process.

After this incubation step, the solution may then be clarified to remove unwanted material such as any minor amounts of precipitate.

Similar to as described with Complex I, the solution may then be concentrated and spray dried before further use.

**Method of use**

The mineral-protein complex may be added to food and beverage products; or as the base for any product to be consumed orally, in order to provide a source of an essential mineral. This means that animals may get the essential minerals from alternative sources to help reach the intake required for optimum health.

Most preferably, the mineral that is to be provided via the food product is selected from iron, zinc, copper, manganese, magnesium, selenium or chromium.

*Outline of further advantages of the present invention*

- Liquid milk and food products may be fortified with minerals using the
complexes of the present invention. In the case of iron for example, sodium ferredetate and ferrous bisglycinate are available to do this, but it is very expensive to do on a large scale.

- Ease of mixing powder of the complexes with food/beverages. A flowable powder with low bulk density will mix better than high density iron fortificants e.g. sodium ferredetate and ferrous bisglycinate.

- A wide range of mineral (e.g. iron) fortification in beverages is possible without affecting taste, colour and shelf-life.

- Complex I is milk based, and complex II is preferably casein based. This means the complexes may be applicable to standardised dairy foods with no substantial regulatory challenges.

- The complexes I and II are soluble at physiological pH (6.6 to 6.9).

- Unlike the prior art, the complexes of the present invention advantageously do not undergo substantial aggregation, are not prone to precipitation, are heat stable at up to 90 °C for 30 min or even 140 °C for 5 seconds, are translucent and/or are highly stable. This temperature stability exceeds that achieved by existing products, such as ferrous bisglycinate.

- For iron (as an example), a creamish-white coloured powder may be produced by the method of manufacture. This may give a transparent solution at 25% daily requirement levels as listed in example 3

- The complexes will not cause changes in pH of milk or other neutral products.

- The complexes may be mixed with liquid and powdered food products. Concentrated small batches may be prepared and added to bulk milk
without sophisticated mixing equipment.

- The manufacturing process of Complex I may be performed continuously from milk.

**BRIEF DESCRIPTION OF DRAWINGS**

Further aspects of the present invention will become apparent from the following description which is given by way of example only and with reference to the accompanying drawings in which:

- **Figure 1** A preferred method for manufacture of complex I;
- **Figure 2** A preferred method for manufacture of complex II;
- **Figure 3** Effect of iron addition on the levels of soluble protein;
- **Figure 4** Effect of iron addition on the levels of soluble iron;
- **Figure 5** Effect of iron addition on the turbidity of sodium caseinate solution;
- **Figure 6** Photograph 1 to illustrate the advantages of complex II;
- **Figure 7** Photograph 2 to illustrate the advantages of complex II;
- **Figure 8** Effect on protein solubility upon iron fortification using complex I;
- **Figure 9** Effect on iron solubility upon iron fortification using complex I; and
- **Figure 10** Effect of protein solubility upon exogenous phosphorus addition.
BEST MODES FOR CARRYING OUT THE INVENTION

Example 1: Physico-chemical properties and composition of 70% Calcium removed milk (used for Complex I)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Greenish translucent liquid</td>
</tr>
<tr>
<td>pH</td>
<td>6.80</td>
</tr>
<tr>
<td>Total solids</td>
<td>10% w/w</td>
</tr>
<tr>
<td>Viscosity (20°C)</td>
<td>1.28 Pascal seconds (50 shear)</td>
</tr>
<tr>
<td>% Ca removed</td>
<td>70% w/w</td>
</tr>
<tr>
<td>Heat stability</td>
<td>Heat Stable (90°C for 30 min or 140 °C for 5 seconds)</td>
</tr>
<tr>
<td>Protein</td>
<td>3.12% w/w</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>96% w/w</td>
</tr>
<tr>
<td>Zeta potential ( 100 X dilution)</td>
<td>-45.58</td>
</tr>
<tr>
<td>Z-avg diameter value</td>
<td>173 nm</td>
</tr>
<tr>
<td>Ca</td>
<td>300 – 350 mg/kg</td>
</tr>
<tr>
<td>Mg</td>
<td>40.7 mg/kg</td>
</tr>
<tr>
<td>K</td>
<td>2500 mg/kg</td>
</tr>
<tr>
<td>P</td>
<td>940 mg/kg</td>
</tr>
<tr>
<td>Na</td>
<td>642 mg/kg</td>
</tr>
</tbody>
</table>
Example 2: Physico-chemical properties and composition of an exemplary soluble mineral protein complex from a milk-derived liquid source.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Yellowish liquid</td>
</tr>
<tr>
<td>pH</td>
<td>6.80</td>
</tr>
<tr>
<td>Total solids</td>
<td>10% w/w</td>
</tr>
<tr>
<td>Viscosity (20°C)</td>
<td>1.33 Pascal seconds (50 shear)</td>
</tr>
<tr>
<td>% Ca removed</td>
<td>70% w/w</td>
</tr>
<tr>
<td>Heat stability</td>
<td>Heat Stable (90°C for 30 min or 140 °C for 5 seconds)</td>
</tr>
<tr>
<td>Protein</td>
<td>3.10% w/w</td>
</tr>
<tr>
<td>Soluble protein</td>
<td>93% w/w</td>
</tr>
<tr>
<td>Zeta potential (100 X dilution)</td>
<td>-48</td>
</tr>
<tr>
<td>Z-avg diameter value</td>
<td>120 nm</td>
</tr>
<tr>
<td>Ca</td>
<td>300 – 350 mg/kg</td>
</tr>
<tr>
<td>Mg</td>
<td>40.7 mg/kg</td>
</tr>
<tr>
<td>Fe</td>
<td>1675 mg/kg</td>
</tr>
<tr>
<td>Fe/Protein Ratio%</td>
<td>3.3%</td>
</tr>
<tr>
<td>K</td>
<td>2500 mg/kg</td>
</tr>
<tr>
<td>P</td>
<td>2000 mg/kg</td>
</tr>
<tr>
<td>Na</td>
<td>1400 mg/kg</td>
</tr>
</tbody>
</table>
Example 3: Examples to illustrate the amount of each complex needed to achieve maximum iron fortification levels according to RDI’s.

The table below outlines existing permission for iron fortification in different foods.

<table>
<thead>
<tr>
<th>Food</th>
<th>Reference quantity</th>
<th>Maximum claim per reference quantity (% RDI)</th>
<th>Quantity of Iron-protein complex 1 or 2 powder to be added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of Iron in powder</td>
<td>-</td>
<td>-</td>
<td>1.8% 7.5%</td>
</tr>
<tr>
<td>Biscuits containing not more than 200g/kg fat &amp; 50g/kg sugar</td>
<td>35 g</td>
<td>3.0 mg (25%)</td>
<td>166 mg 40 mg</td>
</tr>
<tr>
<td>Cereal Flours</td>
<td>35g</td>
<td>3.0 mg (25%)</td>
<td>166 mg 40 mg</td>
</tr>
<tr>
<td>Bread</td>
<td>50 g</td>
<td>3.0 mg (25%)</td>
<td>166 mg 40 mg</td>
</tr>
<tr>
<td>Pasta</td>
<td>35g uncooked</td>
<td>3.0 mg (25%)</td>
<td>166 mg 40 mg</td>
</tr>
<tr>
<td>Extracts of meat, vegetables or yeast</td>
<td>5g</td>
<td>1.8mg (15%)</td>
<td>100 mg 24 mg</td>
</tr>
<tr>
<td>Analogues of meat derived from legumes</td>
<td>100 g</td>
<td>3.5mg (30%)</td>
<td>194 mg 100 mg</td>
</tr>
<tr>
<td>Formulated Beverages</td>
<td>600 ml</td>
<td>3.0 mg (25%)</td>
<td>166 mg 47 mg</td>
</tr>
<tr>
<td>Formulated meal replacements</td>
<td>One meal servings</td>
<td>4.8 mg (40%)</td>
<td>266 mg 64 mg</td>
</tr>
<tr>
<td>Formulated supplementary foods</td>
<td>One serving</td>
<td>6.0 mg (50%)</td>
<td>333 mg 80 mg</td>
</tr>
<tr>
<td>Formulated supplementary sports foods</td>
<td>One day quantity</td>
<td>12 mg (100%)</td>
<td>666 mg 160 mg</td>
</tr>
</tbody>
</table>
The recommended daily intake (RDI) for iron is 12 mg.

The table also illustrates the amount of each complex which is required to be added (in powder form) to the food to achieve the maximum iron fortification for each product. This exemplified the versatility of the complexes and their use. It also shows the advantage of being able to load higher amounts of iron into the complexes, as less powder is needed to achieve high iron fortification in the food.

**Example 4: Effect of phosphorus addition to the complex**

Figures 3 to 5 illustrate the effect of adding phosphorus to the complex.

Figure 3 shows how the protein solubility is affected as iron levels increase from 1 to 20 mM (equivalent to 6.9% iron). As illustrated, as phosphorus levels are increased from 0 mg/kg through to 2000 mg/kg, the protein solubility is significantly improved, regardless of the increase in iron loading.

Figure 4 similarly shows the effect on solubility of the iron in a sodium caseinate solution. Again, as phosphorus levels are increased, the solubility of iron is improved significantly.

Figure 5 illustrates the advantages of the invention, wherein an increase in turbidity indicates a reduction in stability due to the formation of small particulates/precipitates. As the amount of phosphorus is increased, the turbidity can be reduced close to baseline even upon loading up to 25 mM (6.9%) iron, indicating that all the protein is remaining in a soluble and stable form.

Based on these preliminary results, the inventors foresee that a particularly optimal level of mineral loading (e.g. iron) may be about 15 mM (4%). This may provide the best balance between stability and loading for many commercial applications. However, increases beyond 15 mM (4%) are clearly possible and may be viable for
particular applications as discussed in Example 3 above.

**Example 5:** Visual representation of effect of phosphorus addition to sodium caseinate

Figures 6 and 7 visually illustrate how addition of phosphorus improves the solubility and stability of the complex. Even when the iron is loaded up to 25 mM, the composition remains in solution. Without the phosphorus, the protein and/or iron precipitates even at lower levels of iron (5-10 mM).

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope of the appended claims.

**Example 6:** Testing of other minerals

**Zinc**

We have compared the effect of zinc sulphate addition on the precipitation of proteins in sodium caseinate using our technology.

Upon addition of zinc sulphate to sodium caseinate solution (2% protein), not more than 5 mM of zinc could be added without gross precipitation of proteins at pH 6.8. However, as exemplified with the concept of complex II, we could add 18 mM of zinc to the sodium caseinate (2% protein solution) without any precipitation of proteins.

**Copper**

The sodium caseinate solution (2% protein) precipitated upon addition of 1.5 mM copper as copper sulphate. Again using the concept of complex II, we could add 4 mM without noticeable precipitation at pH 6.8.
Example 7: Heat stability and sensory analysis of Complex I

An iron-protein complex according to "Complex I" was added to whole milk powder (WMP) at a level equivalent to 37.5mg iron per 100g WMP. This was then reconstituted to 12% solids using water, equivalent to natural milk. This provided a final iron concentration of 4.5 mg per 100ml serving, equivalent to 25% of the RDA for menstruating women or 56% of the RDA for adult males and postmenopausal women.

The reconstituted WMP was then pasteurised at 75 °C for 15 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 7 days. It was then assessed for functional and sensory characteristics as follows:

- Fortified milk and un-fortified control milk had no difference in colour as measured by Minolta. Sensory assessment found no difference in colour or taste between the fortified and control products.

- Tea: a tea bag was brewed for 4 min in 180ml boiling water. 20ml cold milk was added and stirred. Sensory assessment found no difference in colour or taste between the tea made with the fortified or un-fortified control milk.

- Dark coffee: 2 scoops ground plunger coffee was brewed for 2 min in 300g boiling water. 20g of this brewed coffee was then added to 50g boiling milk. Sensory assessment found no difference in taste between the dark coffee made with the fortified or un-fortified control milk. However, there was a significant change in colour between the two milks, with the fortified milk causing the coffee to turn a dark grey.

- Milky coffee: 2 scoops ground plunger coffee was brewed for 2 min in 300g boiling water. 20g of this brewed coffee was then added to 100g boiling milk. Sensory assessment found no difference in taste between the milky
coffee made with the fortified or un-fortified control milk. However, there was a significant change in colour between the two milks, with the fortified milk causing the coffee to turn a dark grey.

In an additional study, the reconstituted WMP was UHT processed at 140 °C for 5 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 7 days. Sensory testing on the milk showed a small difference in taste between the fortified and un-fortified control products, but this was not rated as an unpleasant difference. There was no difference in colour. The fortified product could also be added to tea and dark coffee without any differences in taste, although there was a small negative effect on the taste of milky coffee. There were significant colour differences in the coffee products.

Separately, chocolate mix (Nestle Nesquik) was added to the reconstituted WMP at a concentration of 6g Nesquik in 100 g milk. The chocolate-flavoured milks were then pasteurised at 75 °C for 15 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 2 days. Sensory assessment showed a small but acceptable change in colour and no difference in flavour between the fortified and un-fortified control milks. In addition, the chocolate-flavoured milks were UHT processed at 140 °C for 5 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 2 days. Sensory assessment showed a noticeable but acceptable change in colour and no significant difference in flavour between the fortified and un-fortified control milks.

Example 8: Heat stability and sensory analysis of Complex Ii

An iron-protein complex according to "Complex II" was added to whole milk powder (WMP) at a level equivalent to 37.5mg iron per 100g WMP. This was then reconstituted to 12% solids using water, equivalent to natural milk. This provided a final iron concentration of 4.5 mg per 100ml serving, equivalent to 25% of the RDA.
for menstruating women or 56% of the RDA for adult males and postmenopausal women.

The reconstituted WMP was then pasteurised at 75 °C for 15 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 7 days. It was then assessed for functional and sensory characteristics as follows:

- Fortified milk and un-fortified control milk had no difference in colour as measured by Minolta. Sensory assessment found no difference in colour or taste between the fortified and control products.

- Tea: a tea bag was brewed for 4 min in 180ml boiling water. 20ml cold milk was added and stirred. Sensory assessment found no difference in colour or taste between the tea made with the fortified or un-fortified control milk.

- Dark coffee: 2 scoops ground plunger coffee was brewed for 2 min in 300g boiling water. 20g of this brewed coffee was then added to 50g boiling milk. Sensory assessment found no difference in colour or taste between the dark coffee made with the fortified or un-fortified control milk.

- Milky coffee: 2 scoops ground plunger coffee was brewed for 2 min in 300g boiling water. 20g of this brewed coffee was then added to 100g boiling milk. Sensory assessment found no difference in taste between the milky coffee made with the fortified or un-fortified control milk. There was only a very slight difference in colour between the products, but this was not noticeable unless they were directly compared.

In an additional study, the reconstituted WMP was UHT processed at 140 °C for 5 seconds, filled into plastic bottles (1 litre) and stored at 4 °C for 7 days. Sensory testing on the milk showed a small difference in taste between the fortified and un-fortified control products, but this was not rated as an unpleasant difference. There
was no difference in colour. The fortified product could also be added to tea, milky
coffee and dark coffee without any differences in taste, although there were
significant colour differences in the coffee products.

Separately, chocolate mix (Nestle Nesquik) was added to the reconstituted WMP at
a concentration of 6g Nesquik in 100 g milk. The chocolate-flavoured milks were
then pasteurised at 75 °C for 15 seconds, filled into plastic bottles (1litre) and
stored at 4 °C for 2 days. Sensory assessment showed a small but acceptable
change in colour and no difference in flavour between the fortified and un-fortified
control milks. In addition, the chocolate-flavoured milks were UHT processed at
140 °C for 5 seconds, filled into plastic bottles (1litre) and stored at 4 °C for 2
days. Sensory assessment showed a noticeable but acceptable change in colour
and no significant difference in flavour between the fortified and un-fortified control
milks.
WHAT WE CLAIM IS:

1. A mineral-protein complex, the complex including
   a) a mineral; and
   b) a protein

characterised in that the protein is derived from a milk source and wherein the milk source has a ratio of protein to calcium is equal to or above 45:1, and wherein the mineral-protein complex includes over 1% w/w mineral bound to protein.

2. The complex as claimed in claim 1 wherein the lactating animal is a mammal.

3. The complex as claimed in either claim 1 or 2 wherein the milk source is cows milk.

4. The complex as claimed in any one of the above claims wherein the ratio of protein to calcium in the milk source is equal to or above 58:1.

5. The complex as claimed in any one of the above claims wherein the ratio of protein to calcium in the milk source is approximately between 58:1 and 640:1.

6. The complex as claimed in any one of the above claims wherein approximately 70% of the calcium (w/v) has been removed from the milk source.

7. The complex as claimed in any one of the above claims wherein the mineral-protein complex includes between 1% to 20% w/w mineral bound to protein.

8. The complex as claimed in any one of the above claims wherein the
mineral-protein complex includes between 1% to 7% w/w mineral bound to protein.

9. The complex as claimed in any one of the above claims wherein the mineral is iron.

10. The complex as claimed in claim 9 wherein the iron is ferric and/or ferrous salts of iron.

11. The complex as claimed in any one of the above claims wherein the protein from the milk source is selected from casein, whey protein and their individual fraction, mixture and derivatives of same.

12. The complex as claimed in any one of the above claims wherein the protein is casein.

13. The complex as claimed in any one of the above claims wherein the mineral-protein complex includes exogenously added phosphorus.

14. The complex as claimed in any one of the above claims wherein the mineral-protein complex includes an amount of phosphorus to provide a ratio of protein to phosphorus in the milk source of up to 8:1.

15. The complex as claimed in claim 14 wherein the mineral-protein complex includes an amount of phosphorus to provide a ratio of protein to phosphorus in the milk source of up to 6.25:1.

16. The complex as claimed in any one of the above claims wherein the mineral-protein complex is used as a food additive or ingredient within a nutritional beverage product, food product, therapeutic/pharmaceutical composition or animal feed composition.

17. A method of manufacturing a mineral-protein complex, the resulting
complex including a mineral and a protein, and wherein the protein is
derived from a milk source having a ratio of protein to calcium equal to or
above 45:1 and wherein the mineral-protein complex includes over 1% w/w
bound mineral,

the method including the step of:

a) adding the mineral to the milk source.

18. The method as claimed in claim 17 wherein the milk source is a milk in
liquid form selected from the group consisting of whole milk, skimmed milk,
low lactose milk, ultrafiltration retentate concentrated milk and combinations
thereof.

19. The method as claimed in claims 17 to 18 wherein the milk is stirred, or in
case of powder source, is dissolved in an amount of water, and mixed at a
temperature between 2-95°C.

20. The method as claimed in any one of claims 17 to 19 wherein the method
includes removing an amount of calcium from the milk source.

21. The method as claimed in claim 20 wherein the amount of calcium removed
from the milk source utilises an ion exchange step.

22. The method as claimed in claim 21 wherein the ion exchange step utilises a
weakly acidic cation exchange resin of K+ form.

23. The method as claimed in claim 21 or 22 wherein the amount of resin used
is 0.1-80% w/v.

24. The method as claimed in any one of claims 22 to 23 wherein the amount of
resin used is 0.1 to 50% w/v.

25. The method as claimed in any one of claims 17 to 24 wherein the method
includes reducing the amount of calcium in the milk source to a ratio of protein to calcium equal to or greater than 58:1.

26. The method as claimed in any one of claims 17 to 25 wherein the method includes reducing the amount of calcium in the milk source to a ratio of protein to calcium of approximately 106:1.

27. The method as claimed in any one of claims 17 to 26 wherein the method includes removing the amount of calcium in the milk source by at least 50% w/w and up to 100% w/w of the calcium from the milk source.

28. The method as claimed in any one of claims 17 to 27 wherein the pH is maintained between 6.0 to 8.5 using at least one pH regulator.

29. The method as claimed in any one of claims 17 to 28 wherein the pH is maintained at approximately 6.5 to 7.5.

30. The method as claimed in any one of claims 17 to 29 wherein the calcium is removed whilst retaining the temperature between approximately 2-10°C.

31. The method as claimed in any one of claims 17 to 30 wherein the method includes adding phosphorus to the low calcium milk source.

32. The method as claimed in any one of claims 17 to 31 wherein phosphorus is added to the milk source to provide a ratio of protein to phosphorus between 64:1 to 8:1.

33. The method as claimed in any one of claims 17 to 32 wherein phosphorus is added to the milk source solution to provide a ratio of protein to phosphorus between 30:1 to 8:1.

34. The method as claimed in any one of claims 17 to 33 wherein the
temperature is maintained between 2-10°C when the mineral is added.

35. The use of a mineral-protein complex as claimed in any one of claims 1 to 16 for the manufacture of a fortified product, to help an animal achieve the dietary mineral intake required for optimum health.

36. An ingredient for use in fortified products to help an animal meet its mineral requirements for optimum health, the ingredient consisting of a mineral-protein complex as claimed in any one of claims 1 to 16.

37. The ingredient as claimed in claim 36 wherein the mineral is selected from iron, zinc, copper, manganese, magnesium, selenium, chromium, or combinations thereof.

38. A mineral-protein complex as herein described and with reference to Figure 1 and Example 3 in Best Modes section.

39. A method of manufacturing a mineral-protein complex as herein described and with reference to Figure 1 and Example 3 in Best Modes section.

40. A mineral-protein complex including an exogenously added mineral and a protein, wherein the mineral-protein complex is soluble in a solution at a physiological pH between 6.6 to 6.9 characterised in that the complex includes exogenous phosphorus.

41. The complex as claimed in claim 40 wherein the protein is a phosphoprotein.

42. The complex as claimed in claim 41 wherein the phosphoprotein is casein.

43. The complex as claimed in any one of claims 40 to 42 wherein the casein containing compound is sodium caseinate, potassium caseinate,
ammonium caseinate, lactic casein and/or derivatives or fractions of caseins.

44. The complex as claimed in any one of claims 40 to 43 wherein the mineral is iron.

45. The complex as claimed in any one of the claims 40 to 44 wherein the iron is ferric iron.

46. The complex as claimed in any one of the claims 40 to 45 wherein the mineral-protein complex includes above 1% w/w mineral bound to protein.

47. The complex as claimed in any one of the claims 40 to 46 wherein the mineral-protein complex includes between 1% to 20% w/w mineral bound to protein.

48. The complex as claimed in any one of the claims 40 to 47 wherein the mineral-protein complex includes between 1% to 9% w/w mineral bound to protein.

49. A method of manufacturing a mineral-protein complex including an exogenously added mineral and a protein, wherein the mineral-protein complex is soluble in a solution at a physiological pH between 6.6 to 6.9 wherein the method is characterised by the steps of

a) adding exogenous phosphorus to the protein; and

b) adding the exogenous mineral to the protein to form the complex.

50. The method as claimed in claim 49 wherein the protein used is a casein containing compound.
51. The method as claimed in either of claims 49 to 50 wherein the casein containing compound used in the method is sodium caseinate, potassium caseinate, ammonium caseinate, lactic casein and/or derivatives and fractions of caseins.

52. The method as claimed in any one of claims 49 to 51 wherein the method including dissolving the protein in water to form a solution.

53. The method as claimed in any one of claims 49 to 52 wherein the protein concentration in the solution is configured to be between 1 - 12.5% w/v.

54. The method as claimed in any one of claims 49 to 53 wherein the phosphorus is added to the protein solution prior to the addition of the mineral component.

55. The method as claimed in any one of claims 49 to 54 wherein the source of phosphorus is K$_2$HPO$_4$.

56. The method as claimed in any one of claims 49 to 55 wherein an amount of phosphorus is added to the protein solution such that the ratio of protein to phosphorus is between 5:1 to 130:1.

57. The method as claimed in any one of claims 49 to 56 wherein the ratio of protein to phosphorus is between 7:1 to 90:1.

58. The method as claimed in any one of claims 49 to 57 wherein the mineral is added to the mixture resulting from step a).

59. The method as claimed in any one of claims 49 to 58 wherein the mineral is iron.

60. The method as claimed in claim 59 wherein the iron is ferric iron.
61. The method as claimed in either claim 59 or 60 wherein the ratio of protein to iron is between 200:1 to 2:1.

62. The method as claimed in any one of claims 59 to 61 wherein the ratio of protein to iron is between 100:1 to 7:1.

63. The method as claimed in any one of claims 59 to 62 wherein after step b), the mineral component, protein and phosphorus are mixed for a period of time at 2-10°C.

64. A use of a mineral-protein complex as claimed in any one of claims 40 to 48 in the manufacture of a fortified product to help an animal achieve its dietary mineral intake required for optimum health.

65. The use as claimed in claim 64 wherein the mineral used is selected from iron, zinc, copper, manganese, magnesium, selenium or chromium.

66. An ingredient for use in fortified products, to help an animal achieve the dietary mineral intake required for optimum health, the ingredient consisting of a mineral-protein complex as claimed in any one of claims 40 to 48.

67. The ingredient as claimed in claim 66 wherein the mineral is selected from iron, zinc, copper, manganese, magnesium, selenium, chromium, or combinations thereof.

68. A mineral-protein complex as herein described and illustrated with reference to Examples 2 and 3, and Figure 3 (B) the Best Modes section.

69. A method of manufacturing the mineral-protein complex as herein described and illustrated with reference to Figure 2 in the Best Modes section.
**Figure 1**

Skimmed Milk/powder/ultrafiltration retentate/low lactose SMP

- **Mixing for 5 min at 2-10°C**

- **Ion Exchange process: Batch or continuous**
  
  *(K⁺ form Weakly acidic cation exchange resin)*

  - **Temperature 2 – 10°C**

  - **Mixing/column passing till 70% of initial Ca is removed**

  - **pH Adjustment to pH 6.8 (5°C) using 1 M NaOH**

  - **Clarification**

  - **Calcium Removed Milk (CaRM)**

  - **Addition of K₂HPO₄ [optional]**

  - **Addition of 0.5M FeCl₃·6H₂O with process pH maintained between 6.5 - 7.5**

  - **Additional volume adjustment to 15 ml (inclusive of Fe solution + 1M NaOH + Water)**

  - **Stirring for 30 min at 2 – 10°C**

  - **Clarification**

  - **Concentration and Spray Drying**
Figure 2

Sodium/Potassium, Ammonium Caseinate/Lactic casein

Dissolving and mixing in water at 50°C for 30 min (3.0%) w/w)

Chilling the solution to 2°C

Addition of K₂HPO₄ salt solution

pH adjustment to pH 6.8 (5°C) using 0.5 M HCL (optional)

Addition of 0.5M FeCl₃.6H₂O with process pH maintained between 6.7 - 6.9

Additional volume adjustment with water if required

Stirring for 30 min at 2 - 10°C

Clarification

Concentration and Spray Drying
Effect of Iron addition on the level of soluble protein in a sodium caseinate solution (2% protein) containing (●) 0 mg/kg, (○) 500 mg/kg, (▼) 1000 mg/kg and (△) 2000 mg/kg of phosphorus. Samples were centrifuged at 500 x g for 10 min at 20°C.
Figure 4

Effect on iron additional on the levels of soluble iron in a sodium caseinate solution (2% protein) containing (●) 0 mg/kg, (○) 500 mg/kg, (▼) 1000 mg/kg and (▲) 2000 mg/kg of phosphorus. Samples were centrifuged at 500 x g for 10 min at 20°C.
Figure 5

Effect of iron addition on the turbidity of sodium caseinate solution (2% protein) containing (•) 0 mg/kg, (o) 500 mg/kg, (▼) 1000 mg/kg and (Δ) 2000 mg/kg of phosphorus.
Figure 3: Interaction of various combinations of sodium caseinate, iron and phosphorus (A) 2.5% sodium caseinate and 20mM FeCl₃ (B) 2.5% sodium caseinate, 20mM FeCl₃ and 1500 mg K₃HPO₄ (complex 2) (C) 20mM FeCl₃ and 1500 mg K₃HPO₄
Figure 7

Iron added to sodium caseinate without phosphorus

Iron added to sodium caseinate using with 2000 mg/kg phosphorus
Figure 8

Soluble protein

% of total

0  5  10  15  20  25  30
Fe (mM)

- Milk
- 70% Calcium depleted milk

8/10
Figure 9

Soluble iron

Solubilisation of iron-protein complex upon ultracentrifugation (100,000 x g, 1 hr at 20°C)
Effect of exogeneous phosphorus addition on soluble protein (●), iron (■) and Z-average diameter values (●) of 30 mM Fe added 70% Ca depleted milk
INTERNATIONAL SEARCH REPORT

PCT/NZ2013/000109

A. CLASSIFICATION OF SUBJECT MATTER

A23C 9/152 (2006.01)  A23J 3/10 (2006.01)  A23L 1/304 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

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

EPDOC, WIPO, CAPplus, FSTA, FROSTI, Medline, Google Scholar (Keywords: milk, casein, iron, complex, low calcium, phosphate and like terms)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*  Citation of document, with indication, where appropriate, of the relevant passages  Relevant to claim No.

Documents are listed in the continuation of Box C

[X] Further documents are listed in the continuation of Box C  [X] See patent family annex

Date of the actual completion of the international search  Date of mailing of the international search report
30 August 2013  30 August 2013

Name and mailing address of the ISA/AU  Authorised officer

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Form PCT/ISA/210 (fifth sheet) (July 2009)
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<td>US 2003/0165597 A1 (AUGUSTIN et al.) 04 September 2003 Abstract; examples; table 2, 8; [0014], [0038], [0069].</td>
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<td>Y</td>
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<td>X</td>
<td>WO 2000/05 1446 A1 (SOCIETE DES PRODUITS NESTLE S.A.) 08 September 2000 Abstract; examples 1, 5; page 5, line 29; page 5, lines 33-34.</td>
<td>40-54, 56-67</td>
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<tr>
<td>A</td>
<td>Abstract</td>
<td>9, 10, 37</td>
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Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 38, 39, 68, 69
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
   See Supplemental Box

3. ☐ Claims Nos:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
 ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 ☐ No protest accompanied the payment of additional search fees.
Supplemental Box

Continuation of Box II
The claims do not comply with Rule 6.2(a) because it/they rely on references to the description and/or drawings.

Continuation of Box III
This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-37 are directed to a mineral-protein complex with over 1% w/w mineral and protein derived from a low calcium milk source, and the method of manufacture and use thereof. The feature of a low calcium milk source and binding over 1% w/w of the mineral is specific to this group of claims.

- Claims 40-67 are directed to a mineral-protein complex with exogenous phosphorus that is soluble at pH 6.6 to 6.9, and the method of manufacture and use thereof. The feature of adding exogenous phosphorus and solubility at pH 6.6 to 6.9 is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is a mineral-protein complex.

However this feature does not make a contribution over the prior art because it is disclosed in:
WO 2000/05 1446 A1 (SOCIETE DES PRODUITS NESTLE S.A.) 08 September 2000

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied a posteriori.
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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End of Annex