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(54) **Titre : Fortified savoury food concentrate.**

(57) **Abrégé :**

The present invention relates to a savoury food concentrate comprising iron salt and a process to produce the same. It is therefore an aim of the present invention to provide a glutamate containing savoury food concentrate which comprises an iron salt, wherein the amount of off-color which appears upon storage of the food concentrate is reduced, preferably wherein off-coloring is absent. It is therefore a further object of the present invention to improve the bioavailability of iron in food concentrates. It was found that a composition comprising an iron salt and a further non-iron phosphate salt, provides reduced discolouration in food concentrate compositions comprising glutamate, and also provides improved bioavailability of the iron.

17461

ORIGINAL

« DEMANDE DE BREVET D'INVENTION »

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Fortified Savoury Food Concentrate

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Fortified savoury food concentrate

The present invention relates to a savoury food concentrate comprising iron salt and a process to produce the same.

Background of the invention

Savoury food concentrates, like seasoning cubes are a well known concentrate food product which is normally dissolved before consumption. It is normally dissolved in water or a dish, in this way giving rise to a bouillon, a soup, a sauce, a gravy or a seasoned dish. A savoury food concentrate cube normally comprises salt and often glutamate, which, among other functions, contribute to the taste impact on the food product which results upon dilation of the bouillon cube. A bouillon cube has been recognised in the field as a relatively cheap and convenient way to provide iron to the population. This is especially relevant for a population which suffers from a lack of iron in its diet, as can be observed in several developing countries. Iron-fortified bouillon cubes have been described in the art.

It is especially desired by present day consumers that the iron is not only present in a composition, but is also taken up by the body; this is generally referred to in the art as bioavailability.

WO2009/068378 discloses a bouillon cube comprising, based on the weight of the bouillon cube: 30-70 percent wt. NaCl, 10-45 percent wt. monosodium glutamate, and at least one iron compound of the group of ferric sodium EDTA, reduced iron, ferrous lactate, ferric citrate, ferric pyrophosphate, ferrous sulphate monohydrate, ferric ammonium citrate brown, in such an amount that the bouillon cube comprises an amount of Fe^{2+} and/or Fe^{3+} taken together of from at least 0.01 percent wt. and less than 2 percent wt, based on the weight of the bouillon cube. This document aims at reduction of off-color in the food product resulting after dilution of the fortified cube.

WO2010/086192 discloses a dry savoury food concentrate comprising NaCl, an iron ion selected from the group consisting of $Fe2^{+}$ and Fe^{3+} and mixtures thereof, which iron ion is derived from an added iron compound which is dissolvable in an aqueous solution. This document aims at reduction of staining of the cooking pot during cooking when a fortified food concentrate is used.

The problem of off-color related to iron fortification not only appears on the level of the cooking pot and the ready-to-eat food product which results upon dilution of the food concentrate, for example a seasoning cube, but can also be present in the food concentrate itself. Such an appearance of off-coloring may be observed after a certain time of storage, which can depend on relative air humidity and temperature during transport and storage in the shop and by the consumer at home. It may be perceived that especially in tropical or sub-tropical areas the risk for off-coloring effects is higher than in moderate climates. It is however also the sub-tropical and tropical area where fortified products can show their highest benefit to the population. Off-coloring can be observed for example in the form of dark spots or stains on the surface of the food concentrate, e.g. on the surface of a seasoning cube.

The respective mechanisms which underlie these iron-related off-coloring effects are not clear. It was observed that the appearance of off-color on the food concentrate, was significant when glutamate was present. Without willing to be bound to theory, the presence of glutamate appears to have a negative influence on the appearance of a iron-fortified food concentrate.

It is therefore an aim of the present invention to provide a glutamate containing savoury food concentrate which comprises an iron salt, wherein the amount of off-color which appears upon storage of the food concentrate is reduced, preferably wherein off-coloring is absent.

It is therefore a further object of the present invention to improve the bioavailability of Iron in food concentrates.

It was surprisingly found that a composition comprising an Iron salt and a further non-Iron phosphate salt, provides reduced discolouration in food concentrate compositions comprising glutamate, and also provides improved bioavailability of the Iron.

Summary of the invention

Accordingly, the present invention provides a savoury food concentrate comprising:

- NaCl,
- glutamate,
- iron salt, further comprising
- phosphate salt, not being an iron phosphate.

In a second aspect, the invention provides a process to provide a savoury food concentrate according to the invention, the process comprising the steps of:

a) preparing a mixture comprising
   - NaCl,
   - iron salt,
   - glutamate, and further comprising
   - phosphate salt, not being an iron phosphate,

b) packaging.

In a third aspect the invention provides the use of a concentrate according to the invention for preparing a bouillon, a soup, a sauce, a gravy or a seasoned dish.

In a fourth aspect the invention provides the use of a phosphate salt to prevent discolouration of food concentrates comprising an iron salt in the presense of glutamate.

In a fifth aspect the invention provides the use of a phosphate salt to enhance the bioavailability of iron in a food composition.

Detailed description of the invention

The present invention provides a savoury food concentrate comprising NaCl, glutamate, iron salt, and a phosphate salt, not being an iron phosphate.

<u>NaCl</u>

The present invention relates to a savoury food concentrate. The savoury character of the food concentrate is at least partly created by the presence of NaCl. The concentrate of the invention therefore comprises NaCl, preferably in an amount of from 10 to 70 wt%, preferably of from 30 wt% to 70 wt%, more preferably of from 40 wt% to 65 wt%, even more preferably of from 45 wt% to 60 wt%, based on the weight of the food concentrate. It can be preferred that in addition to NaCl, optionally a potassium salt is present, like for example KCl, for example to replace part of the NaCl by potassium salt. Potassium salt, preferably KCl, can preferably be present in a ratio potassium salt, preferably KCl, to NaCl of from 1:10 to 1:1, preferably of from 1:10 to 1:2.

Glutamate

The savoury food concentrate of the invention comprises glutamate. Although glutamate contributes to the savoury character of the food concentrate, without willing to be bound to theory, it appeared that the presence of glutamate is associated with off-coloring in the iron-fortified food concentrate. The type and intensity of off-coloring can depend on the type and amount of iron salt present in the food concentrate and the time and circumstances of storage. In general, the off-coloring results in a darker appearance of the food concentrate, that can go from brownish, to dark brown, even towards black color of the food concentrate. Especially iron sulphate can provide off color in the form of dark speckles, developing towards general dark coloring after prolonged storage time. In general, the amount of off color tends to increase with increased storage time.

Preferably, the invention relates to a food concentrate wherein glutamate comprises one of the group consisting of mono sodium glutamate, potassium glutamate, glutamic acid and mixtures thereof. More preferably, glutamate comprises mono sodium glutamate. Glutamate is preferably present in an amount of from 0.5 to 45 wt%, more preferably in an amount of from 1 to 35 wt%, even more preferably in an amount of from 5 to 30 wt%, most preferably in an amount of from 7 to 20 wt%, based on the weight of the food concentrate. Preferably the savoury food concentrate comprises monosodium glutamate, which is preferably present in an amount of from 0 to 45 wt%, more preferably in an amount of from 1 to 35 wt%, even more preferably in an amount of from 5 to 30 wt%, most preferably in an amount of from 7 to 20 wt%, based on the weight of the food concentrate.

Iron salt

The savoury food concentrate of the invention comprises iron salt. The iron salt preferably comprises, more preferably is, an iron salt selected from the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof.

Even more preferably, the iron salt comprises, even more preferably is an iron salt selected from the group consisting of iron phosphate, ferrous sulphate and mixtures thereof. Iron phosphate is a term known to the skilled artisan and comprises the group of salts comprising one or more iron atoms and one or more phosphate groups. It comprises for example ferric orthophosphate, ferric pyrophosphate, or ferrous orthophosphate.

Even more preferably, the iron salt comprises, even more preferably is, ferric pyrophosphate or ferrous sulphate or a mixture thereof.

Even more preferably the iron salt comprises ferric pyrophosphate, even more preferably is ferric pyrophosphate.

Iron salt is preferably present in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the food concentrate.

Preferably, the food concentrate comprises an iron salt selected from the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the food concentrate. It can be preferred that an iron salt is selected from this list, to be present in the food concentrate, which iron salt individually can be present in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the food concentrate.

More preferably, the food concentrate comprises an iron salt selected from the group consisting of ferric pyrophosphate, ferrous sulphate and a mixture thereof in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the food concentrate.

Even more preferably, the food concentrate comprises ferric pyrophosphate in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the food concentrate.

Phosphate salt

The savoury food concentrate of the invention comprises a phosphate salt. The phosphate salt is not an iron-phosphate salt, as iron-phosphate salt is separately categorised in this description under 'iron salt'. Indeed, in case the iron salt is an iron phosphate, the food concentrate comprises in addition to said iron phosphate another phosphate salt that is not an iron phosphate.

The phosphate salt preferably comprises a phosphate salt, more preferably is a phosphate salt, selected form the group consisting of orthophosphate salt, diphosphate salt, triphosphate salt, polyphosphate salt and mixtures thereof. The phosphate salt preferably comprises a salt selected from the group consisting of Na-phosphate salt, K-phosphate salt, Ca-phosphate salt and Mg-phosphate salt. More preferably, the phosphate salt comprises Na-phosphate salt.

Preferably Na-phosphate salts comprise Na_3PO_4, Na_2HPO_4, NaH_2PO_4, sodium pyrophosphate, sodium triphosphate, sodium polyphosphate and mixtures thereof.

Preferably, K-phosphate salts comprise K_3PO_4, K_2HPO_4, KH_2PO_4, potassium

pyrophosphate, potassium triphosphate, potassium polyphosphate and mixtures thereof. The phosphate salt preferably comprises a salt selected from the group consisting of Na3PO4, Na2HPO4, NaH2PO4, K3PO4, K2HPO4, KH2PO4, calcium phosphate, magnesium phosphate, sodium pyrophosphate, sodium triphosphate, potassium pyrophosphate, potassium triphosphate, sodium polyphosphate, potassium polyphosphate and mixtures thereof. More preferably, the phosphate salt comprises a salt selected from the group consisting of Na3PO4, Na2HPO4, NaH2PO4, K3PO4, K2HPO4, KH2PO4, sodium pyrophosphate, sodium triphosphate, potassium pyrophosphate, potassium triphosphate and mixtures thereof. Most preferably, the phosphate salt comprises Na-pyrophosphate. The phosphate salt is preferably Na-pyrophosphate.

Phosphate salt, not being an iron phosphate, preferably Na-phosphate salt, is preferably present in an amount of from 0.03 to 20 wt%, more preferably from 0.07 to 10 wt%, even more preferably of from 0.1 to 5 wt%, even more preferably of from 0.2 to 2 wt%, based on the weight of the food concentrate.

It is especially preferred if the iron salt comprises ferric pyrophosphate and the phosphate salt comprises sodium pyrophosphate.

For stability, the molar ratio of iron salt, preferably iron pyrophosphate, to phosphate salt (not being an iron-phosphate), preferably Na-pyro phosphate, is preferably of from 0.1 to 10 (i.e from 1:10 to 10:1), preferably of from 0.25 to 5 (i.e. from 1:4 to 5:1), most preferably of from 0.5 to 2 (i.e. from 1:2 to 2:1).

For improved bioavailability, the molar ratio of phosphate salt to ionic iron, the ratio is preferably of from 1:1 to 20:1, more preferably 1:1 to 10:1, still more preferably 1:1 to 6:1, wherein it is noted that the effect is difficult to measure at a ratio of less than 1:1 and that above 10:1 the effect is constant and does not further improve; however, the addition of more phosphate is also not detrimental to the bioavailability.

Binder

The food concentrate of the invention can comprise a binder, for example to maintain the desired texture and/or shape of the food concentrate. A binder is especially preferred when the food concentrate is in the form of a cube or tablet. Preferably, the binder comprises one selected from the group consisting of fat, polysaccharide, sugar, and mixtures thereof. Sugar

preferably comprises monosaccharide. Polysaccharide preferably comprises starch, gums or mixtures thereof.

Fat

The food concentrate of the invention preferably comprises fat. This is preferred especially when the food concentrate is a cube or tablet, such as a bouillon cube or tablet, a soup cube or tablet or a seasoning cube or tablet. Fat can function as a binder. Fat preferably comprises a fat selected from the group consisting of chicken fat, beef fat, vegetable fat, pork fat and mixtures thereof. Fat is preferably present in an amount of from 1 to 35 wt%, preferably of from 5 to 30 wt%, more preferably of from 10 to 20 wt%. Especially if the food concentrate is an extruded food concentrate, like for example an extruded cube or tablet, also known in the field as 'pasty' cube, the fat is preferably present in an amount of from 10 to 35 wt%, more preferably of from 15 to 30 wt%, most preferably of from 17 to 25 wt%. Especially if the food concentrate is a pressed cube or tablet or a roller-formed cube or tablet, the fat is preferably present in an amount of from 1 to 20 wt%, preferably of from 5 to 15 wt%, most preferably of from 7 to 12 wt%. However, it might be preferred that the food concentrate has a low fat level, for example of lower than 15 wt%, for example of between 0.1 and 15 wt%., more preferably of lower than 10 wt%, for example of between 0.1 and 10 wt%., even more preferably lower than 5 wt, for example of between 0.1 and 5 wt%, or lower than 2 wt% for example of between 0.1 and 2 wt%, or even lower than 1 wt%., for example of between 0.1 and 1 wt%. These low fat levels might be preferred for example in case of low-fat cubes or tablets or in case of water-based granules or powders, for example bouillon-, soup-, or seasoning granules or powder.

Starch

The food concentrate may preferably comprise starch. Starch is preferably present in an amount of from 0.1 to 15 wt%, 2 to 15 wt%, more preferably of from 5 to 12 wt%, most preferably of from 7 to 10 wt%. Starch can function as a filler. It may further contribute to the mouth feel of the food product resulting upon dilution of the food concentrate. Starch is preferably selected from the group consisting of corn starch, tapioca starch, pea starch, potato starch and mixtures thereof. The starch is preferably non-gelatinised starch. It might be preferred however, that starch comprises gelatinised starch, especially when the starch is used as a binder, a preferred amount of non-gelatinised starch, based on the weight of the food concentrate, can be of from 0.1 to 10 wt%.

Water

The food concentrate of the present invention is preferably a dry food concentrate. Although some water can be present in the food concentrate, it comprises preferably less than 10 wt% of total water, more preferably less than 5 wt%, for example from 1 to 10 wt% or from 2 to 5 wt% of total water, based on the weight of the food concentrate. Total water includes water which may be present in the other ingredients of the food concentrate.

The water activity of the food concentrate is preferably of between 0.1 and 0.6, more preferably of between 0.15 and 0.4, most preferably of between 0.2 and 0.3.

As is commonly understood in the art, salts as described in the present document may, at least partly, dissolve in the food concentrate, especially when water is present. "Salt", like NaCl, glutamate, iron salt and phosphate salt includes the dissolved or partly dissolved or dissociated form of these respective salts. For example, the part of the total amount of added NaCl, which turns into the dissolved form in the food concentrate, contributes to the total amount of NaCl in the food concentrate.

Taste imparting components

It can be preferred that the food concentrate comprises plant particles, for example particles from herbs or from vegetable. The plant particles are preferably present in an amount of from 0.1 wt% to 20 wt%, more preferably of from 0.5 wt% to 10 wt%, even more preferably of from 1 to 5 wt%, or they can be preferred to be present in an amount of from 0.5 to 2 wt% (dry weight of the plant particles based on weight of the food concentrate).

The food concentrate of the present invention preferably comprises flavours. Flavours can be present for example in an amount of for example 0.1 wt% to 10 wt%, more preferably of from 0.5 wt% to 8 wt%, (dry weight of the flavours based on weight of the food concentrate). Flavours are preferably selected from the group consisting of vegetable flavour, meat flavour and mixtures thereof. Meat flavour preferably comprises flavours selected from the group consisting of chicken flavour, fish flavour, beef flavour, pork flavour, lamb flavour, and mixtures thereof. Vegetable flavour preferably comprises spices.

The food concentrate might contain extract of beef. The food concentrate may contain extract of yeast, alternatively to, or in addition to extract of beef. Extract of beef and extract of yeast can individually be present in an amount of from 1 to 10 wt%, preferably in an amount of from 2 to 7 wt%, based on the weight of the food concentrate. It can be preferred that the total

amount of beef extract and yeast extract taken together is present in an amount of from 1 to 10 wt%, preferably in an amount of from 2 to 7 wt%, based on the weight of the food concentrate.

The savoury food concentrate is preferably in the form of a cube, a tablet, a granule, or a powder. It is more preferably a cube or a tablet, most preferably a pressed cube or tablet. A cube or tablet is preferably between 2 and 30 grams, preferably of between 3 and 20 grams, more preferably of between 4 and 12 grams. A cube and tablet are terms used in this description inter-exchangeable, as common in the field, and do not refer necessarily to geometrically defined structures. It can be preferred however that the cube is a regular figure wherein height, width and height have the same size. It can be preferred that the tablet is an oblong wherein the longest dimension (length) and the second-longest dimension (width) 1:1 to 1:3, preferably of between 1:1 to 1:2. The longest dimension (length) and the shortest dimension (height) preferably relate to each other as 1:2 to 1:6.

The savoury food concentrate is preferably a concentrate selected from the group consisting of a bouillon concentrate, a soup concentrate, a gravy concentrate, a sauce concentrate and a seasoning concentrate.

Preferably, the present invention relates to a savoury food concentrate in the form of a cube or a tablet comprising glutamate, NaCl, iron salt and a phosphate salt, wherein

- NaCl is present in an amount of from 10 to 70 wt%,
- Glutamate is present in an amount of from 0.5 to 45%,
- Iron salt is present in an amount of from 0.03 to 2 wt%, and wherein the iron- salt is selected from the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof, preferably wherein the iron- salt is selected from the group consisting of iron phosphate, ferrous sulphate and mixtures thereof, most preferably wherein the iron salt is selected from the group consisting of ferrous sulphate, ferric pyrophosphate and mixtures thereof.
- phosphate salt, not being an iron phosphate, is present in an amount of from 0.03 to 20 wt%, and wherein the phosphate salt is selected from the group consisting of Na_3PO_4, Na_2HPO_4, NaH_2PO_4, K_3PO_4, K_2HPO_4, KH_2PO_4, calcium phosphate, magnesium phosphate, sodium pyrophosphate, sodium triphosphate, potassium

pyrophosphate, potassium triphosphate, sodium polyphosphate, potassium polyphosphate and mixtures thereof.

The savoury food concentrate is preferably packaged. It can be packaged for example in a wrapper, a sachet, a box or a tub.

Process

In a further aspect, the present invention relates to a process to provide a food concentrate according to the invention, the process comprising the steps of:

a) preparing a mixture comprising:
- NaCl,
- glutamate
- iron salt, preferably selected from the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof,,
- phosphate salt, not being an iron phosphate, wherein the phosphate salt is preferably selected from the group consisting of Na3PO4, Na2HPO4, NaH2PO4, K3PO4, K2HPO4, KH2PO4, calcium phosphate, magnesium phosphate, sodium pyrophosphate, sodium triphosphate, potassium pyrophosphate, potassium triphosphate, sodium polyphosphate, potassium polyphosphate and mixtures thereof.

b) packaging,

to result in a food concentrate.

In step a) a mixture is provided comprising NaCl, iron phosphate and phosphate salt. The mixture can further comprise fat, water, starch, flavour.

NaCl is preferably added to the mixture of step a) in an amount of from 30 wt% to 70 wt%, more preferably of from 40 wt% to 65 wt%, even more preferably of from 45 wt% to 60 wt%, based on the weight of the resulting food concentrate.

Glutamate is preferably added in an amount of from 0.5 to 45 wt%, more preferably in an amount of from 1 to 35 wt%, even more preferably in an amount of from 5 to 30 wt%, most preferably in an amount of from 7 to 20 wt%, based on the weight of the resulting food concentrate. Preferably glutamate is added in the form of monosodium glutamate. Mono sodium glutamate is preferably added in an amount of from 0.5 to 45 wt%, more preferably in

an amount of from 1 to 35 wt%, even more preferably in an amount of from 5 to 30 wt%, most preferably in an amount of from 7 to 20 wt%.

Iron salt is preferably added to the mixture of step a) in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the resulting food concentrate. Fe-pyro phosphate and/or ferrous sulphate is preferably added in an amount of from 0.03 to 2 wt%, more preferably of from 0.07 to 1 wt%, based on the weight of the resulting food concentrate.

For stability, Iron salt, preferably iron phosphate and/or ferrous sulphate, more preferably iron pyrophosphate and/or ferrous sulphate, and phosphate salt, not being an iron-phosphate, preferably Na-pyro phosphate, are preferably added to result in a molar ratio of iron phosphate, preferably iron pyrophosphate, to phosphate salt, not being an iron-phosphate, preferably Na-pyro phosphate, of from 0.1 to 10, preferably of from 0.25 to 5, most preferably of from 0.5 to 2, as present in the resulting food concentrate.

For improved bioavailability, the molar ratio of phosphate salt to ionic iron, the ratio is preferably of from 1:1 to 20:1, more preferably 1:1 to 10:1, still more preferably 1:1 to 6:1, wherein it is noted that the effect is difficult to measure at a ratio of less than 1:1 and that above 10:1 the effect is constant and does not further improve; however, the addition of more phosphate is also not detrimental to the bioavailability.

Phosphate salt, not being an iron phosphate, preferably Na-phosphate salt, is preferably added in an amount of from 0.03 to 20 wt%, more preferably from 0.07 to 10 wt%, even more preferably of from 0.1 to 5 wt%, even more preferably of from 0.2 to 2 wt%, based on the weight of the resulting food concentrate.

Water can be added to the mixture of step a). If added, it is preferably added in an amount of from 0.5 to 4 wt%, preferably in an amount of from 1 to 3 wt%, based on the weight of the total resulting food concentrate.

If fat is present, it is preferably added to the mixture of step a) in the total amount of from 1 to 35 wt%, preferably of from 5 to 30 wt%, more preferably of from 10 to 20 wt%. Especially if the food concentrate is an extruded food concentrate, like for example an extruded cube, also known in the field as 'pasty' cube, the fat is preferably added in a total amount of from 10 to 35 wt%, more preferably of from 15 to 30 wt%, most preferably of from 17 to 25 wt%.

Especially if the food concentrate is a pressed cube or a roller formed cube, the fat is preferably added in a total amount of from 1 to 20 wt%, preferably of from 5 to 15 wt%, most preferably of from 7 to 12 wt%.

The process according to the invention may further comprise the step of shaping the mixture resulting from step a). Shaping preferably comprise compressing or extruding the mixture of step a) to result in a cube or tablet. These technologies are known in the art and can be carried out for example by a press from Fette™ or Bonals™, to obtain a hard cube, also known as pressed cube or by an extruder from Corazza™, to obtain an extruded cube, also known as soft cube or as pasty cube. The compressing can alternatively be carried out by roller compaction. Shaping can comprise roller formation. These shaping techniques have been described in the art.

Shaping may comprise one of granulation, agglomeration, or roller compaction, for example in case granules or pellets are desired. These technologies are known in the art. Granulation is preferably carried out in a basket granulator, as known in the art.

Accordingly, the process of the present invention preferably comprises shaping, wherein shaping comprises a technique selected from the group consisting of compression, extrusion, roller compacting, granulation, agglomeration and mixtures thereof.

The process of the invention further comprises the step of packaging. The mixture resulting from step a) or the shaped mixture, in case the process comprises the step of shaping the mixture resulting from step a), is packaged, for example to allow transportation and/or dosing of the product. Preferably packaging comprises packaging in a packaging selected from a wrapper, a jar, a box, a tub, a sachet and mixtures thereof.

The present invention further relates to the use of a concentrate according to the invention for preparing a bouillon, a soup, a sauce, a gravy or a seasoned dish.

The invention describes a savoury food concentrate comprising glutamate, NaCl, iron salt and further comprising a phosphate salt, wherein said phosphate salt is different from an iron phosphate. By the present invention the appearance of off-color in glutamate containing savoury food concentrates which are fortified with an iron salt can be significantly reduced.

The invention will now be exemplified by the following, non limiting Examples:

Examples

Materials and Methods – Corn starch 'C*GEL LM03411' was obtained from Cargill UK Ltd. Yeast extract 'Gistex Xtra Light' was obtained from DSM Food Specialties (min.weight MSG 3.0 weight%). Ferrous sulphate, ferric pyrophosphate and iron phosphate were obtained from Dr. Paul Lohmann GmbH KG. Sodium pyrophosphate was obtained from BK Guilini. Trisodium citrate was obtained from Merck. Sodium dicalcium EDTA was obtained from Akzo. Sodium polyphosphate was obtained from Riedel-de-Haen. All other phosphates were obtained from Sigma Aldrich.

Preparation of seasoning cubes - All the materials, with the exception of the palm stearate and the iron salt are weighed together in a plastic jar and mixed with a mixer (Kenwood Chef Premier KMC650) for 1 minute at speed setting 4. The palm stearate is molten by placing a container containing it in a hot water bath and is added to the mixture when liquid, after which the mixture is mixed for 1 minute at speed setting 6. The iron salt is added and again the mixture is mixed for 1 minute at speed setting 6. 4 Grams of this mixture at a time are transferred in the pressing block of an Instron press (Instron 5567) and the cube is pressed at 5 kN. This procedure is repeated for each cube.

Test procedure – Off-color formation is analysed in an accelerated off-color test. Two cubes are put on a plastic holder and placed in a 100 ml glass jar. 1 Gram of water is added in the jar in such a way that the cube does not come into direct contact with the water. This procedure simulates typical storage conditions of commercial products, where the water content of seasoning cubes increases over time, but in an accelerated fashion. The jars are closed with an lid and placed in an oven at 40°C for the accelerated test. Results are given after three weeks. For example, after 3 weeks comparative samples containing ferrous sulphate are very dark, and comparative samples with iron pyrophosphate show significant discoloration. The off-color formation was representative for discolouration observed after longer storage times in real-life.

Colour measurements - Off-color formation was analysed by a color measurement as known in the art. All colour measurement have been performed by using a DigiEye Imaging system from VeriVide Ltd. From photographs under controlled and calibrated conditions the L*a*b* values were determined. The colour difference ΔE was determined using the formula: ΔE = square root of $((L_1^*-L_0^*)^2 + (a_1^*-a_0^*)^2 + (b_1^*-b_0^*)^2)$. Where L_1^*, a_1^* and b_1^* are the colour values for the sample, and the L_0^*, a_0^* and b_0^* are the values for the reference relative to which the colour value is expressed. In the examples mentioned hereafter all colour

differences are expresses as ΔE relative to the formulation without any iron added. A high ΔE value represents a relatively high amount of off-color. All values given represent average of duplo measurements.

Example 1

In this experiment, a seasoning cube without iron – composition 1 (table 1) – was compared to a seasoning cube with FeSO4, iron pyrophosphate and a combination of iron pyrophosphate and 1 equivalent of sodium pyrophophate (composition 2, 3 and 4 in table 1). In each case, the total iron content was kept constant and the formulation was adapted in the amount of sodium chloride.

Ingredient	**Compar. comp. 1 (no iron salt)**	**Compar. comp. 2 (Fe pyrophosphate)**	**Comp. 3 (Iron pyrophosphate + Na pyrophosphate)**
NaCl	53	52.6	52.2
Sucrose	15.5	15.5	15.5
Corn starch	5.7	5.7	5.7
Palm stearate	7	7	7
MSG	14	14	14
Yeast	3	3	3
Herbs and spices	1.8	1.8	1.8
Ferric pyrophosphate		0.4	0.4
Sodium pyrophosphate			0.4
total	**100**	**100**	**100**

Table 1: compositions of Example 1; all numbers in weight%. Comp.=composition. Compar. Comp.= comparative composition.

The seasoning cubes were prepared, tested in an accelerated test and color measurements were performed, as described in the Materials and Methods section above. The results are given in table 2 in L*a*b* and corresponding ΔE value relative to the reference sample without iron.

	L*	**a***	**b***	**Delta E**
Compar. comp. 1	62.6	11.8	58.1	0

Compar. comp. 2	55.17	12.4	47.1	13.3
composition 3	61.6	11.6	53.8	4.4

Table 2: L*a*b* values of the seasoning cubes measured in the accelerated test after 21 days. Delta E values relative to composition 1.

Off color was observed as a darker, brownish appearance of the cube. The addition of sodium pyrophosphate significantly reduces the off-colour formation of the cube after storage.

Example 2

Following the same procedures as described in Example 1, ferric pyrophosphate in combination with various phosphates were tested. The compositions are given in table 3, the results in table 4.

Ingredient	**Compar. Comp. 1**	**Compar. Comp. 2**	**Comp. 3**	**Comp. 4**	**Comp. 5**
NaCl	53.0	52.6	52.3	51.9	52.4
Sucrose	15.5	15.5	15.5	15.5	15.5
Corn starch	5.7	5.7	5.7	5.7	5.7
Palm stearate	7.0	7.0	7.0	7.0	7.0
MSG	14.0	14.0	14.0	14.0	14.0
Yeast	3.0	3.0	3.0	3.0	3.0
Herbs and spices	1.8	1.8	1.8	1.8	1.8
Ferric pyrophosphate		0.4	0.4	0.4	0.4
Sodium dihydrogen phosphate			0.3		
sodium triphosphate				0.7	
Sodium polyphosphate					0.2
total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

Table 3: compositions of Example 2; all numbers in weight%. Comp.=composition. Compar. Comp.= comparative composition.

	L*	**a***	**b***	**Delta E**
Compar. Comp.1	62.6	11.8	58.1	0.0

Compar. Comp. 2	55.2	12.4	47.1	13.3
Comp. 3	59.4	10.6	49.0	9.7
Comp. 4	64.2	10.4	54.0	4.6
Comp. 5	62.4	10.6	55.0	3.3

Table 4: L*a*b* values of the seasoning cubes measured in the accelerated test after 21 days. Delta E values relative to composition 1.

Off color was observed as a darker, brownish appearance of the cube. As can be seen from the results, the addition of the phosphates significantly limited the colour formation in the samples: sodium dihydrogen phosphate decreases the colour formation in the cube with ferric pyrophosphate significantly, while triphosphate and polyphosphate decrease it to an even larger extent.

Example 3

Following the same procedures as described in Example 1, various iron salts in combination with phosphates were tested. The compositions are given in table 5, the results in table 6.

Ingredient	**Compar. Comp. 1**	**Comp. 2**	**Comp. 3**	**Comp. 4**	**Comp.5**
NaCl	53.0	52.4	52.5	52.5	52.6
Sucrose	15.5	15.5	15.5	15.5	15.5
Corn starch	5.7	5.7	5.7	5.7	5.7
Palm stearate	7.0	7.0	7.0	7.0	7.0
MSG	14.0	14.0	14.0	14.0	14.0
Yeast	3.0	3.0	3.0	3.0	3.0
Herbs and spices	1.8	1.8	1.8	1.8	1.8
Ferric phosphate		0.2	0.2	0.2	0.2
Trisodium phosphate			0.3		
Disodium hydrogenphosphate				0.3	
Sodium dihydrogenphosphate					0.2
Sodium pyrophosphate		0.4			
total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

Comp.=composition. Compar. Comp.= comparative composition.

Table 5: compositions of Example 3; all numbers in weight%.

	L*	a*	b*	Delta E
Compar. Comp. 1	62.6	11.8	58.1	0.0
Comp. 2	61.3	11.1	54.1	4.3
Comp. 3	57.9	11.2	49.4	9.9
Comp. 4	57.7	11.1	48.6	10.7
Comp. 5	57.2	11.6	48.0	11.5

Table 6: L*a*b* values of the seasoning cubes measured in the accelerated test after 21 days. Delta E values relative to composition 1.

Off color was observed as a light brownish appearance of the cube. As can be seen from the results, all samples showed less discoloration than the samples with ferric pyrophosphate (see Example 1). The combination of ferric phosphate with sodium pyrophosphate gave the least discoloration.

Example 4

Following the same procedures as described in Example 1, the effect of FeSO4 in combination with sodium pyrophosphate was tested. The compositions are given in table 7, the results in table 8.

Ingredient	**Compar. Comp. 1**	**Compar. Comp. 2**	**Comp. 3**	**Compar. Comp. 4**	**Comp. 5**
NaCl	53.0	52.8	52.4	52.6	52.2
Sucrose	15.5	15.5	15.5	15.5	15.5
Corn starch	5.7	5.7	5.7	5.7	5.7
Palm stearate	7.0	7.0	7.0	7.0	7.0
MSG	14.0	14.0	14.0	14.0	14.0
Yeast	3.0	3.0	3.0	3.0	3.0
Herbs and spices	1.8	1.8	1.8	1.8	1.8
Ferrous sulphate		0.2	0.2		
Ferrous lactate hydrate				0.4	0.4
Sodium pyrophosphate			0.4		0.4
total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

Table 7: compositions of Example 3; all numbers in weight%. Comp.=composition. Compar. Comp.= comparative composition.

	L*	a*	b*	Delta E
composition 1	62.6	11.8	58.1	0.0
composition 2	36.7	5.2	8.0	56.8
composition 3	58.1	10.9	48.6	10.6
composition 4	52.5	11.2	43.8	17.5
composition 5	55.5	12.3	47.4	12.8

Table 8: L*a*b* values of the seasoning cubes measured in the accelerated test after 21 days. Delta E values relative to composition 1.

As can be seen from the results, ferrous sulphate on its own (comparative composition 2) gave severe discolouration. Off color was observed as dark speckles and darker color of the cube. Also ferrous lactate on its own resulted in off color formation. (comparative composition 4). Off color was observed in this case as a darker, brownish appearance of the cube. The presence of sodium pyrophosphate (composition 3 and 5) resulted in a significant reduction of off color formation.

Comparative example 5

As a comparative example, the use of sodium citrate and sodium calcium EDTA was tested in combination with ferric pyrophosphate and compared to ferric pyrophosphate in combination with sodium pyrophosphate. The procedure as described in Example 1 was followed. The compositions are given in table 9 and the results in table 10.

Ingredient	**Compar. comp. 1**	**Compar. comp. 2**	**Compar. comp. 3**	**Compar. comp. 4**
NaCl	53.0	52.6	52.1	50.7
Sucrose	15.5	15.5	15.5	15.5
Corn starch	5.7	5.7	5.7	5.7
Palm stearate	7.0	7.0	7.0	7.0
MSG	14.0	14.0	14.0	14.0
Yeast	3.0	3.0	3.0	3.0
Herbs and spices	1.8	1.8	1.8	1.8
Ferric pyrophosphate		0.4	0.4	0.4
Ingredient	**Compar. comp. 1**	**Compar. comp. 2**	**Compar. comp. 3**	**Compar. comp. 4**

Disodium calcium EDTA			0.5	
Trisodium citrate				1.9
total	**100.0**	**100.0**	**100.0**	**100.0**

Table 9: compositions of Example 3; all numbers in weight%. Comp.=composition. Compar. Comp.= comparative composition.

	L*	**a***	**b***	**Delta E**	**Off color**
Compar. comp. 1	62.6	11.8	58.1	0.0	
Compar. comp. 2	55.17	12.4	47.1	13.3	brownish
Compar. comp. 3	47.6	13.1	20.9	40.1	dark brown
Compar. comp. 4	50.0	11.5	40.2	21.9	dark brown

Table 10: L*a*b* values of the seasoning cubes measured in the accelerated test after 21 days. Delta E values relative to composition 1.

As can be seen from the results, the use of the two most commonly used sequestrants did not give similar improvement of the colour stability of the seasoning cube: in fact, in both cases much more discolouration was observed as compared with the sample with ferric pyrophosphate only, demonstrating the unique properties of phosphate salts, as exemplified in the previous examples 1 to 4.

Example 6: Bioavailability

An example composition of bouillon cubes containing FeSO4 made at Unilever R&D Vlaardingen was made according to table 11 below. is presented in Table 1. The mix is produced in a quantity large enough to ensure homogeneity and from the mix a specific weight (nominal 4000 mg) is pressed in a dye to a cube approximately 1 x 1 x 1 cm (5 kN Instron press force).

Table 11: Sample composition of test cubes with FeSO4 (weight %)

Sample name:	**HBR120013**	**HBR120014**
ingredient:	Whole mix	Whole mix + FeSO4
NaCl	51.2	51.2

sugar	15	15
corn starch	5.6	5.6
fat	7.0	7.0
MSG	14	14
I&G	0.2	0.2
Yeast	3	3
Curcuma	0.35	0.35
onion	0.8	0.8
garlic	1	1
bayleaf	0.1	0.1
Fe(II)SO4		0.65

Table 12: Sample composition HBR series of test cubes with FePP (weight %)

Experiment sample compositions	HBR12059-02	
sample name	Full formulation + FePP + NaPP	
ingredient:	target amount	weighed actual (g)
NaCl	129.42	129.43
Sugar	37.92	37.96
corn starch	14.16	14.16
Fat	17.69	17.66
MSG	35.39	35.38
I&G	0.51	0.58
Yeast	7.58	7.59
Curcuma	0.88	0.88
Onion	2.02	2.05
Garlic	2.53	2.54
Bayleaf	0.25	0.25
micronized Fe (III)Pyrophosphate (Fe4P6O21)(FePP)	0.95	0.96

The amount of salt (NaCl) was corrected when Iron pyrophosphate (FePP) and Sodium pyrophosphate (NaPP) were added, the total weight of one cube remains 4 g.

Two consecutive sets of experiments have been executed within URDV with these example formats to explore the effect of NaPP on in-vitro Iron bioavailability further.

The first set of experiments focused on in-vitro bioavailability of iron of several bouillon cubes formats for the Nigerian market in order to rank prototypes to be used in an in-vivo study.

The experimental protocol included both dissolution / digestion and iron uptake experiments using human colonic adenoma carcinoma (Caco-2) cells. Gastrointestinal dissolution / digestion tests are simple tests to determine the ability of a compound to dissolve in gastrointestinal fluid. This method is well known by the skilled person. The experiments are based on simulating gastrointestinal digestion, by exposure of food products and meals to gastric-like conditions (low pH and gastric enzymes) followed by intestinal-like conditions (neutralization of pH and incubation with pancreatic enzymes and bile salts). Subsequently, the digested meal or food product is dialysed and the soluble/ionic/dialyzable fractions (containing solutes and small molecules) are collected for determination of iron content.

For non-heme iron this set-up first solubilises all iron at pH 2.0, mimicking the gastric conditions. Then, during the intestinal phase at neutral pH, some of the iron will form complexes with components from the (food) matrix. The ionic iron diffuses into a dialysis membrane (< 8000 g/mol) till equilibrium is reached while the complexed iron – with exception of low molecular weight soluble complexes – remains on the outside of the membrane. The ionic iron solution is collected from the membrane pouch and used for analysis to calculate the "dialyzable iron fraction", or for further iron uptake experiments with intestinal cells.

To simulate the absorption of iron by intestinal cells after the gastrointestinal digestion, the model uses in-vitro gastrointestinal digestion techniques (see above) coupled with uptake of iron by Caco-2 cell monolayers. After 21 days of culture under adequate conditions, Caco-2 cells form a monolayer that can be used as model of the intestinal epithelium in absorption experiments. If exposed to iron in the ionic form, Caco-2 cells synthesise ferritin as response to iron uptake. The ferritin amount is proportional to the iron content in the cell culture medium. Ferritin can be measured via a commercially available ELISA. Therefore the measurement of cellular ferritin is a good indicator of the iron absorbed by the cell (ref: Glahn, R.P., Lee, O.A., Yeung, A. et al.; Caco-2 cell ferritin formation predicts nonradiolabeled food iron availability in an in vitro digestion/Caco-2 cell culture model; Journal of Nutrition; 1998, Vol. 128, no.9, p. 1555-1561).

Many published studies indicate that these combined digestion and cell models are useful to understand and rank iron uptake from food or meal formats. An international expert panel (ref: Fairweather-Tait, S.J., Lynch, S., Hotz, C. et al.; The usefulness of in vitro models to predict the bioavailability of iron and zinc: A consensus statement from the HarvestPlus expert consultation; International Journal for Vitamin and Nutrition Research; 2005, Vol. 75, no.6, p. 371-374) has reviewed several in-vitro methodologies and concluded that the combined use of digestion and Caco-2 cells are a suitable approach in this field. This in-vitro approach was more recently critically reviewed and the conclusion of the expert panel confirmed (ref: Sandberg, A.S.; The use of Caco-2 cells to estimate fe absorption in humans - a critical appraisal; International Journal for Vitamin and Nutrition Research; 2010, Vol. 80, no.4-5, p. 307-313).

For ranking purposes of prototypes it is enough to perform only the dissolution / digestion part which predicts the amount of bioavailable iron which is available to be taken up by the intestinal cells. However, we have chosen to determine the in-vitro bioavailability of iron in the bouillon cubes in the first series with the complete setup since the combination of iron and NaPP was never tested in the iron uptake experiments and we would like conformation of the iron bioavailability results.

Materials & Methods

Products (first set of experiments)

Bouillon cubes were manually prepared and the amount of iron and sodium pyro-phosphate added to the base formulation (see above) are shown in Table 3. Total of NaCl is corrected for the FePP and NaPP amount to remain at a total weight of each cube = 4 g.

Table 13 Bouillon cubes prepared to study the in-vitro bioavailability of iron from bouillon cubes – series 1 (the amount of iron per cube was set to 3.5 mg, 15% of the RDA, assuming 3 cubes would be shared in a meal for 5p).

Bouillon code	Description
HBR 12061- 1	No Fe
HBR 13003-1	FeSO4 (15% RDA Fe)
HBR 12061-12	FeSO4 + 1eq NaPP (15% RDA Fe)
HBR 13003-2	2xFeSO4 (30% RDA Fe)
HBR 12061-2	Micronized FePP (15% RDA Fe)

HBR 12059-16	Micronized FePP + 0.25eq NaPP (15% RDA Fe)
HBR 12059-2	Micronized FePP + 1eq NaPP (15% RDA Fe)
HBR 12045-14	Micronized 2xFePP (30% RDA Fe)
HBR 12050-12	Micronized 2xFePP + 2x NaPP (30%RDA Fe)

To prepare bouillon with an iron level high enough to determine at the end of the dissolution / digestion experiments, three bouillon cubes of the same batch were added to 400 ml boiled hot milli-Q water in a plastic beaker. The bouillon cubes were dissolved for 5-10 minutes using an incubator of 50 °C at 150 rpm. The obtained bouillon was gently shaken and divided over 4 plastic containers, 3 used for dissolution / digestion experiments and 1 for total iron determination. The intact cubes where also analysed for their total iron content.

Protocols

Total Iron determination

The total iron content of the cubes was determined with open acid extraction method TE460 followed by Inducted Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). Total iron in the start bouillons was determined with open acid extraction method TE460 and with microwave destruction (TE461) followed by ICP-AES as above. Total iron in the samples after the gastric phase and after the intestinal phase (dialysates) was determined with extraction method TE461 followed by ICP-AES.

Ionic iron determination

Ionic iron (sum of Fe2+ and Fe3+) was determined with the Ferrozine method as described by Viollier et al. (Viollier, E., Inglett, P.W., Hunter, K.; The ferrozine method revisited: Fe (II)/(III) determination in natural waters.; Applied Geochemistry; 2000, Vol. 15, no.6, p. 785-790).

Samples were acidified (50 µl 37% HCl added to 1000 µl dialysate) and overnight stored at 4°C.

Standard ($FeSO_4*7H_2O$) was dissolved in 0.5 M HCl and the concentration was corrected for Fe content (20.09 %). A standard series of 0.06 – 30 mg/l Fe diluted in 0.5M HCl was analysed and the linear function was used to calculate the amount of Fe in the dialysates.

Reducing agent (150 µl of 1.4 M hydroxylamine in 2 M HCl) was added to 100 µl sample or standard. After mixing, the solution was allowed to stand for 30 minutes at room temperature before ferrozine solution (100 µl of 0.01 M 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate in 0.1 M ammonium acetate) and buffer (150 µl of 5 M ammonium acetate adjusted to pH9.5 with ammonium hydroxide (28-30%)) were added. The solution was mixed and 300 µl was transferred to a micro plate and absorption at λ562 nm was read. The ferrozine-iron complex is stable for a least one hour.

Dissolution / Digestion

Bioavailability is defined as the fraction of the total amount of iron present in the cube which goes into solution (as ionic iron) and hence has the potential to be absorbed. Bioavailability of the iron present in the fortified bouillons was determined.

A short description of the in-vitro dissolution / digestion is as follows:

All glassware was incubated overnight in 10 % (v/v) HNO3. On the day of the experiment all glassware were washed 5 times with milli-Q to remove HNO3. With exception of the bouillon product without added iron, all bouillon products (80 ml) were transferred into 100 ml dissolution vessels in the dissolution apparatus (USP dissolution apparatus type II, VanKel VK700) and the pH was adjusted to 2.0. Subsequently, pepsin (0.5 g/l) was added to each vessel, yielding a 90 ml solution of bouillon in simulated gastric fluid at pH 2.0. After 60 minutes incubation at 37 °C with mixing at 100 rpm, samples of the products were taken for total iron determination and simulation of the intestinal phase in an Erlenmeyer flask.

For the simulation of the intestinal phase, a dialysis membrane (Spectra/Por 7 MWCO 8000) filled with a water solution of NaHCO3 was placed in the Erlenmeyer flask. The amount of sodium bicarbonate present in the dialysis membrane was able to adjust the simulated digestion to pH 7.5. After 30 minutes incubation in a water bath at 37 °C and continuous shaking (100 rpm) to raise the pH gradually, a mix of pancreatin (0.4 mg/mL) and bile acids solution (1.25 mg/mL) was added to the flask. The flask was further incubated with the dialysis membrane for another 2 hours in the same water bath at 37 °C with continuous shaking (100 rpm). Hereafter, the dialysis membrane was removed and the content of the membrane (dialysate) was stored in aliquots for determination of ionic dialyzable iron and iron uptake experiments.

Average osmolarity of the bouillon samples after dissolution / digestion was 0.280 ± 0.010 Osmol/kg. Average pH after the intestinal phase was 7.2 ± 0.2.

Calculation

Bioavailability of iron is calculated by using Equation 1:

%bioavailable iron= (ionic dialysable iron)/(total Fe) ×100% (Equation 1)

Iron uptake experiments in Caco-2 cells

In-vitro iron bioavailability was determined. However the used medium to dilute the dialyses samples for Caco-2 cell experiments was changed since the osmolarity of the samples with addition of 10 times concentrated medium was too high and resulted in loose cells.

Short description of the iron uptake experiments in Caco-2 cells is as follows:

Caco-2 cells were seeded in 12-wells plates at a density of 2*105 cells per well (passage 15). The cells were grown in Dulbecco's modified Eagle's medium with 4.5 g/L glucose and L-glutamine (Bio-Whittaker), supplemented with 20 % (v/v) heat-inactivated foetal bovine serum (Gibco), 1 % (v/v) penicillin/streptomycin (Bio-Whittaker) and 1 % (v/v) non-essential amino acids (Gibco). The cells were maintained at 37 °C in an incubator with a 5 % CO2 / 95 % air atmosphere at constant humidity; the medium was changed every 2-3 days. The cells were cultured for 21 days, so that they can form a monolayer of differentiated cells that resembles that of the intestinal mucosa. At this point, cells were used for iron uptake experiments.

On the day of experiment, dialysis fluids were thawed and diluted (3 ml dialysis fluid + 0.4 ml 10 times concentrated Customized Minimum Essential Medium+ (CMEM+) + 0.6 ml mQ). Average osmolarity of the samples as applied to the cells was 0.3386 ± 0.0161 Osmol/kg. Customized 10 times concentrated MEM+ (Osmolarity: 1.3 Osmol/kg) was prepared by mixing 20% (v/v) MEM Amino Acids solution (50x, Sigma-Aldrich), 10 % (v/v) non essential amino acids (Bio-Whittaker), 10% (v/v) MEM Vitamin Solution (100×, Sigma-Aldrich), 5 % (v/v) penicillin / streptomycin solution (Bio-Whittaker), L-glutamine solution (20 mM), glucose (100 g/l), CaCl2*2H2O (18 mM), MgSO4*7H2O (8.14 mM),NaH2PO4 (9.3 mM), PIPES (100 mM), NaHCO3 (15.8 g/L), KOH (4 M for pH correction to pH 7.0), hydrocortisone (40 mg/L), insulin (100 mg/L), selenium (50 µg/L), tri-iodotrionine (340 µg/L), epidermal growth factor (200 µg/L) and milli-Q to a final volume of 100 mL. The concentrated CMEM+ was sterile filtered (0.22 µm) and stored in aliquots at -20°C.

The diluted dialysate samples were sterile filtered (0.22 µm). As positive control, a sample was prepared with 5 µM FeSO4 in MEM+ (osmolarity 0.289 Osmol/kg). After washing the cells twice with 1 mL of 1 x MEM+, 1 mL of the diluted dialysate samples and control was applied in duplicate. Dialysates of day 1, 2 and 3 (5 products) were applied to different 12-wells plates. On a fourth 12-wells plate was product 6 applied. The positive control was applied to each plate.

Exactly 48 hours after the start of the incubation of the dialysates and the control in the incubator, the cell monolayers were harvested for ferritin and protein measurements. For this, the medium covering the cells was removed carefully and the cells were washed twice with 1 mL "rinse" solution (containing NaCl (140 mM), KCl (5 mM) and PIPES (10 mM) adjusted to pH 7.0 with NaOH (4 M); osmolarity 0.301 Osmol/kg). The "rinse" solution was then aspirated and 250 µL of ice-cold milli-Q were added. The plate was wrapped in parafilm and sonicated on ice in a water bath at 4 °C for 15 minutes. After sonication the cells were scraped and collected in Eppendorf tubes. The cell lysates were stored at -20 °C until further use.

Ferritin was measured using a commercial ELISA kit (H-ferritin (human) ELISA kit, 96 assays, Abnova, Taipei city, Taiwan, Catalogue number: KA0211) according to the manufacturer's description (version 4). Wavelengths 620, 450 and 405 nm were read according to the Radim protocol (KP33IW – ferritina iema well - M108 – Rev08 - 10/2007). Total protein was measured with the Bradford assay (Bradford reagent, Sigma-Aldrich) using immunoglobulin G (0 – 0.7 mg/ml, Bio-Rad Protein standard 1 (IgG)) as standard. Cell lysates were 10 times diluted prior analysis and 250 µl Bradford reagent was added to 20 µl diluted sample/standard. This assay was performed at 595 nm.

Caco-2 cell iron uptake results were expressed as nanogram (ng) of ferritin per mg of total protein.

Results and Discussion

Total iron content

The total iron content in the bouillon cubes, start bouillon and after the gastric phase was determined. The results are listed in Table 14. The start bouillon is the dissolved bouillon (3 cubes / 400 ml) as used in the dissolution vessel. The iron content after the gastric phase (8/9 dilution) is also determined. From these figures the solubility at pH 2.0 is calculated (Table

14). At pH 2.0 the solubility of FeSO4 is somewhat better then the solubility of FePP, respectively 106 ± 1.9% and 94 ± 3.7%, but on average all iron is dissolved.

The total iron content in the dialysates of the intestinal phase was also determined and compared to the ionic iron content obtained with a ferrozine assay. The two methods show similar results (Table 15), meaning that all iron that passed the dialysis membrane is ionic iron. The large error bars for 30% RDA FeSO4 are due to the sample of the first dissolution / digestion experiment (day 1). The amount of iron found in the dialysate of day 1 resulted in roughly two times the amount of iron as measured for the second and third experiment. This is measured in both assays.

Table 14. Total iron content determined in bouillon cubes, start bouillon and after gastric phase using ICP-AES

Product	**Fe in cube (mg/kg)**	**Fe in start bouillon (mg/kg)**	**Fe after gastric phase* (mg/kg)**	**Solubility (%) at pH 2.0**
No Fe	4.5	n.d.	n.d.	n.a.
FeSO4 (15% RDA)	760	24.8±0.35	23.4±0.60	106
FeSO4 + 1eq NaPP (15% RDA)	665	26.4±0.57	24.5±0.90	104
2xFeSO4 (30% RDA)	1570	47.1±1.41	45.9±0.07	108
FePP (15% RDA)	790	21.5±0.99	17.9±3.20	94
FePP + 0.25eq NaPP (15% RDA)	1080	22.3±0.64	18.8±1.40	90
FePP + 1eq NaPP (15% RDA)	895	22.7±0.00	20.2±2.42	99
2xFePP (30% RDA)	1610	46.1±0.42	39.5±1.69	94
2xFePP + 2eq NaPP (30%RDA)	1650	53.0±0.35	43.1±5.40	91

* Products are 8/9 diluted at the end of the gastric phase compared to the start bouillon.
n.d. = not determined; n.a. = not applicable

Table 15. Comparison of Dialyzable ionic iron content analysed using ICP-AES (total iron) and ferrozine (ionic iron) methods. Mean ± SD (n=3 dissolution / digestion experiments).

Bouillon format	ICP-AES (mg Fe/kg dialysate)	Ferrozine (mg Fe/kg dialysate)
FeSO4 (15% RDA)	1.33 ± 0.06	1.38 ± 0.12
FeSO4 + 1eq NaPP (15% RDA)	3.20 ± 0.30	3.06 ± 0.17
2 x FeSO4 (30% RDA)	2.20 ± 0.79	2.14 ± 0.75
FePP (15% RDA)	0.43 ± 0.06	0.40 ± 0.09
FePP + 0.25eq NaPP (15% RDA)	0.70 ± 0.17	0.68 ± 0.17
FePP + 1eq NaPP (15% RDA)	1.60 ± 0.17	1.59 ± 0.04
2 x FePP (30% RDA)	0.50 ± 0.10	0.47 ± 0.11
2 x FePP + 2eq NaPP (30% RDA)	2.87 ± 0.25	2.71 ± 0.18

Bioavailability (dissolution data)

Bioavailability is defined as the fraction, or percentage, of the total amount of iron present in the cube which goes into solution (as ionic iron). Only unbound ionic iron is potentially absorbed by the intestinal cells. The bioavailability of iron form the bouillon formats is shown in Table 16.

Table 16. Bioavailable ionic iron (%) of the first bouillon series, mean ± SD, n = 3. The amount of iron per cube was set to 3.5 mg, 15% of the RDA, assuming 5 cubes would be shared in a meal for 3p. Statistic analysis was performed with JMP One way ANOVA with student T-test (comparison for all pairs, $p < 0.05$), different letters indicate significant differences.

sample description	**mean (%)**	**St Dev**	**statistical analysis**
FeSO4 (15% RDA; ref)	14.24	0.97	c,d
FeSO4 + 1eq NaPP (15% RDA)	32.65	2.66	a
2xFeSO4 (30% RDA)	11.92	4.40	d,e
FePP (15% RDA)	6.30	2.12	f,g
FePP + 0.2eq NaPP (15% RDA)	9.24	1.82	e.f
FePP + 0.7eq NaPP (15% RDA)	19.79	0.49	b
2xFePP (30% RDA)	3.19	0.73	g
2xFePP + 2eq NaPP (30%RDA)	16.74	1.56	b,c

FeSO4 (14.2%, shows a better bioavailability than FePP (6.3%), the factor is about 2. Surprisingly however, an increase of the bioavailable ionic iron (%) is seen for bouillon containing FePP + NaPP as well as for bouillon containing FeSO4 + NaPP.
Without wishing to be bound by a theory, this increase seems to be dose-dependent for the amount of NaPP added to the bouillon. More NaPP added to bouillon cubes containing FePP result in higher bioavailable iron (%).

In-vitro iron (Caco-2 cell data)

In-vitro iron is defined as the fraction of bioavailable ionic iron which is capable to enter cells to trigger a physiological cell response. In-vitro iron is expressed by the ferritin formation by Caco-2 cells. Ferritin is the natural intra-cellular storage protein for ionic iron and hence ferritin formation is proportional, but not linear, to cell iron (6). The ferritin formation by Caco-2 cells induced by ionic iron present in the dialysates of the first bouillon series is shown in Table 17.

Bouillons without NaPP with 15 and 30% RDA of a Fe source, give similar amounts of ferritin formation. This could be explained by equal amounts of Fe present in their dialysates (Table 15). Addition of 1 equivalent NaPP to the cubes results in an enhancement of iron response in the cells.

When we compare the bioavailability results (Table 16) with the in-vitro bioavailability results (Table 17) indeed, a similar trend is seen between the plots of both methods. This means that also for bouillon cubes containing NaPP, ranking could be performed on base of bioavailability results.

Table 17. Ferritin formation (ng ferritin/mg protein, mean ± SD, n = 3) by human colonic adenoma carcinoma (Caco-2) cells in response to the ionic iron from dialysates obtained from the in-vitro dissolution / digestion of iron. Statistical analysis was performed with JMP One way ANOVA with student T-test (comparison for all pairs, $p < 0.05$). Different letter indicate significant difference. na = not available.

	mean	SD	statistical analysis
FeSO4 solution, 5 micromol/L, reference	49.40	15.32	--
FeSO4 (15% RDA; ref)	9.62	5.30	c,d
FeSO4 + 1eq NaPP (15% RDA)	61.20	12.18	b

2xFeSO4 (30% RDA)	11.38	no SD, n = 1	c,d
FePP (15% RDA)	7.03	2.09	d
FePP + 0.2eq NaPP (15% RDA)	Na	na	--
FePP + 0.7eq NaPP (15% RDA)	34.59	9.64	c
2xFePP (30% RDA)	8.11	no SD, n = 1	c,d
2xFePP + 2eq NaPP (30%RDA)	88.84	28.08	A

Conclusions

Addition of NaPP to FePP containing food formats tested has a positive, and apparently NaPP dose related effect, on the in-vitro bioavailability of iron. Surprisingly, addition of 1 equivalent NaPP shows also enhancement of bioaccessable iron and in-vitro bioavailability for the tested iron source FeSO4.

Example 7: Iron to phosphate ratio

In this example the effect of the phosphate to iron ratio on in-vitro bioaccessibility is determined at different levels of iron added to a cube format and at different molar ratio's, a series of cube formats were prepared and pressed into cubes as indicated above. An experimental design was chosen to cover a range of sodium pyrophosphate to iron pyrophosphate (NaPP/FePP) molar ratio's at different levels, see Table 8.

Table 18. Experimental design used to determine effect of different levels of FePP (X-axis) and NaPP (Y-axis) formulated into a bouillon cube format (nominal weight 4000 mg; having the same composition as in Example 6) on the in-vitro bioaccessible iron.

code	sample description	mg Fe per cube of 4 g	mg NaPP per cube of 4 g
00	(00) JNJ13037-01	25.1	641
+-	(+-) JNJ13037-02	9.1	1089
-+	(-+) JNJ13037-03	41.2	188
0A	(0A) JNJ13037-04	47.8	640
++	(++) JNJ13037-05	41.1	1093
a0	(a0) JNJ13037-07	25.1	1.4
0a	(0a) JNJ13037-08	2.5	641
A0	(A0) JNJ13037-09	25.1	1278
--	(--) JNJ13037-10	9.3	189
00	(00) JNJ13037-11	25.2	641
00	(00) JNJ13037-01	25.1	641

Table 18a Second series of samples to determine effect of different levels of FePP (X-axis) and NaPP (Y-axis) formulated into a bouillon cube format (nominal weight 4000 mg) on the in-vitro bioaccessible iron.

Bouillon cube	Code	FePP (mg)	NaPP (mg)	EQ NaPP/Fe
1 - JNJ13048-05	low Fe 0 Eq NaPP	5	0	0.25
2 - JNJ13048-01	high Fe 0 Eq NaPP	218	0	0.25
3 - JNJ13049-01	low Fe 0.18 Eq NaPP	5	1	0.42
4 - JNJ13048-02	high Fe 0.18 Eq NaPP	217	47	0.43
5 - JNJ13049-02	low Fe 5 Eq NaPP	5	29	5.08
6 - JNJ13048-03	high Fe 5 Eq NaPP	217	1279	5.22
7 - JNJ13049-03	low Fe 15 Eq NaPP	5	86	14.67
8 - JNJ13048-04	high Fe 7.2 Eq NaPP	218	1863	7.47
9 - JNJ13049-04	low Fe 25 Eq NaPP	5	148	25.22
10 -JNJ13049-05	low Fe 35 Eq NaPP	5	207	35.14
11 -JNJ13049-06	low Fe 45 Eq NaPP	5	267	45.32

It is noted that the molar ratio of NaPP/Iron is defined as equivalents, so the relative amount of NaPP over iron becomes independent of the Iron form. This to exclude the impact of Iron coming from a salt low in Iron content (e.g. iron-gluconate) or a salt high in Iron content (e.g. ferrous sulphate, dried) or even to iron in its pure form (elemental- iron). The experimental details are similar to the first series (above).

Bioaccessibility (dissolution)

The effect of the different levels can be seen in Table 19. Please note that each data point not only reflects the molar ratio in equivalents of NaPP over Iron, but also represents different absolute levels of iron added to the cube format (according to Table 18 and 18a). Despite this wide range in compositional variation, the data shows that already at very low ratio's of NaPP/Fe the positive effect on bioavailable ionic iron is significantly present.

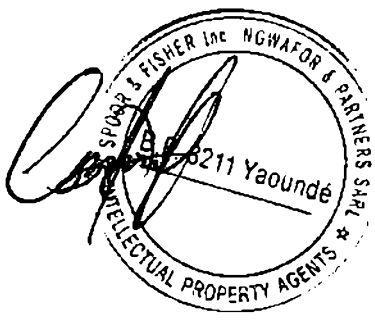
Table 19 Bioavailable ionic iron (%) at different levels of FePP and NaPP in a cube format (4000 mg). KO = Knorr Olympus formulation. Sample codes between brackets (e.g. (0A), refers to the sample codes as in experiment in Table 18.

sample description	Ratio PP/Fe	% Bioavailable ionic iron	Stdev

low Fe 0 Eq NaPP	0.25	28.8	1.9
high Fe 0 Eq NaPP	0.25	2.7	0.3
KO (a0)JNJ13037-07	0.26	4.7	0.2
FePP (15 % RDA)	0.30	6.3	2.1
2xFePP (30% RDA)	0.30	3.2	0.7
low Fe 0.18 Eq NaPP	0.42	19.1	2.2
high Fe 0.18 Eq NaPP	0.43	5.4	0.1
FePP + 0.25eq NaPP (15% RDA)	0.50	9.2	1.8
FeSO4 + 1eq NaPP (15% RDA)	0.97	32.6	2.7
FePP + 1eq NaPP (15% RDA)	1.07	19.8	0.5
2xFePP + 2x NaPP (30%RDA)	1.09	16.7	1.6
KO (-+) JNJ13037-03	1.21	22.0	0.9
KO (0A) JNJ13037-04	3.06	49.3	5.4
KO (--) JNJ13037-10	4.52	52.2	7.2
low Fe 5 Eq NaPP	5.08	46.9	4.8
high Fe 5 Eq NaPP	5.22	59.6	4.4
KO (00)JNJ13037-11	5.60	62.5	12.6
KO (00) JNJ13037-01	5.61	69.2	7.1
KO (++) JNJ13037-05	5.83	68.3	3.6
high Fe 7.2 Eq NaPP	7.47	70.2	0.2
KO (A0)JNJ13037-09	10.95	63.2	8.1
low Fe 15 Eq NaPP	14.67	62.4	6.2
low Fe 25 Eq NaPP	25.22	62.5	3.4
KO (+-) JNJ13037-02	25.40	82.7	4.7
low Fe 35 Eq NaPP	35.14	60.6	3.4
low Fe 45 Eq NaPP	45.32	61.1	5.2
KO (0a)JNJ13037-08	54.78	30.5	4.2

The table above shows that the percentage of bioavailable iron does not substantially increase above a ratio of phosphate to Iron ion of 10:1. Good bioavailability is obtained at a ratio of 1:1 or more. However the level of bioavailable iron remains high above 10:1, The best results are obtained at a ratio of between 1:1 and 6:1. The KO(0a) value is likely to be an outlier, because that was measured on a very low concentration of Iron and a high NaPP concentration in the composition, causing an increased measuring error.

Without wishing to be bound by a theory, it is thought that 6 pyrophosphate molecules may be arranged around one ionic iron atom to form a stable, soluble complex with an overall negative charge, existing at a neutral pH. This is further remarkable, as for other ionic iron forms, the solubility depends strongly on the pH. At neutral pH in aqueous systems, iron is not in solution but forms insoluble (poly)hydroxides.



Claims

1. Savoury food concentrate comprising:
   - NaCl,
   - glutamate,
   - iron salt, further comprising
   - phosphate salt, not being an iron phosphate.

2. Food concentrate according to claim 1, wherein iron salt comprises one of the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof.

3. Food concentrate according to any one of the preceding claims, wherein the phosphate salt comprises a salt selected form the group consisting of orthophosphate salt, diphosphate salt, triphosphate salt, polyphosphate salt and mixtures thereof.

4. Food concentrate according to any one of the preceding claims, wherein the phosphate salt is one of the group consisting of Na3PO4, Na2HPO4, NaH2PO4, K3PO4, K2HPO4, KH2PO4, calcium phosphate, magnesium phosphate, sodium pyrophosphate, sodium triphosphate, potassium pyrophosphate, potassium triphosphate, sodium polyphosphate, potassium polyphosphate and mixtures thereof.

5. Food concentrate according to any one of the preceding claims, wherein the iron salt comprises one of the group consisting of ferric pyrophosphate, ferrous sulphate and mixtures thereof, and the phosphate salt comprises Na-pyrophosphate.

6. Food concentrate according to any one of the preceding claims, wherein the glutamate is selected from one of the group consisting of monosodium glutamate, potassium glutamate, glutamic acid and mixtures thereof.

7. Food concentrate according to any one of the preceding claims, wherein the molar ratio of iron salt to phosphate salt, not being an iron phosphate, is between 1 and 10.

8. Food concentrate according to any one of the preceding claims, wherein the iron salt is present in an amount of from 0.03 to 2 wt%, based on the weight of the food concentrate.

9. Food concentrate according to any one of the preceding claims, wherein the phosphate salt is present in an amount of from 0.03 to 20 wt%, based on the weight of the food concentrate.

10. Food concentrate according to any one of the preceding claims, wherein the food concentrate is in the form of a cube or a tablet.

11. Food concentrate according to any one of the preceding claims, wherein the savoury food concentrate is in the form of a cube or a tablet and wherein

- NaCl is present in an amount of from 10 to 70 wt%,
- iron-salt is present in an amount of from 0.03 to 2 wt%, based on the weight of the food concentrate, and wherein the iron-salt is one of the group consisting of ferrous sulphate, ferrous gluconate, ferrous lactate, ferrous bisglycinate, ferrous fumerate, ferric orthophosphate, ferric pyrophosphate, ferrous tartrate, ferrous succinate, ferrous saccharate, ferrous orthophosphate and mixtures thereof, preferably wherein the iron- salt is selected from the group consisting of iron phosphate, ferrous sulphate and mixtures thereof, preferably is one of the group consisting of ferric pyrophosphate, ferrous sulphate and mixtures thereof.
- phosphate salt, not being an iron phosphate, is present in an amount of from 0.03 to 20 wt%, based on the weight of the food concentrate, and wherein this phosphate salt preferably is one of the group consisting of Na3PO4, Na2HPO4, NaH2PO4, K3PO4, K2HPO4, KH2PO4, sodium pyrophosphate, sodium triphosphate, potassium pyrophosphate, potassium triphosphate and mixtures thereof,
- the glutamate is selected from one of the group consisting of monosodium glutamate, potassium glutamate, glutamic acid and mixtures thereof, preferably comprises mono sodium glutamate,
- wherein the molar ratio of iron phosphate to phosphate salt, not being an iron phosphate, is between 1 and 10.

12. Process to provide a food concentrate according to any one of claims 1 to 11, the process comprising the steps of:

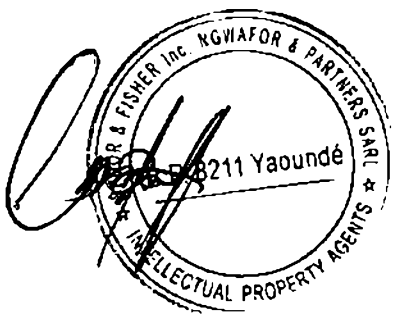
a) preparing a mixture comprising
   - NaCl,
   - iron salt,
   - glutamate, and further comprising
   - phosphate salt, not being an iron phosphate,

b) packaging.

13. Process according to claim 12, further comprising the step of shaping the mixture resulting from step a).

14. Process according to claim 13, wherein the shaping comprises a technique selected from the group consisting of compression, extrusion, roller compacting, granulation, agglomeration and combinations thereof.

15. Use of a concentrate according to any one of claims 1 to11 for preparing a bouillon, a soup, a sauce, a gravy or a seasoned dish.



Abstract

The present invention relates to a savoury food concentrate comprising iron salt and a process to produce the same.
It is therefore an aim of the present invention to provide a glutamate containing savoury food concentrate which comprises an iron salt, wherein the amount of off-color which appears upon storage of the food concentrate is reduced, preferably wherein off-coloring is absent. It is therefore a further object of the present invention to improve the bioavailability of Iron in food concentrates.

It was found that a composition comprising an Iron salt and a further non-Iron phosphate salt, provides reduced discolouration in food concentrate compositions comprising glutamate, and also provides improved bioavailability of the Iron.