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(54) Title: PROCESS FOR THE PREPARATION OF A FOOD ADDITIVE

(57) Abstract: The present invention provides a process for the preparation of an iron-containing food additive which is prepared from plant material comprising chlorophyll. The process comprises the steps of; (a) treating plant matter comprising chlorophyll with an aqueous solution of an acid, and simultaneously or subsequently treating the plant matter with an iron containing compound, (b) neutralizing the matter from step a) with an alkaline material to produce treated plant matter, (c) separating water soluble material from the treated plant matter and (d) drying the treated plant matter to obtain a powder. The food additive produced by the process comprises iron in a highly bio-absorbable form and is convenient to manufacture.



WO 2005/104874 A1

Process for the preparation of a food additive

Technical field

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The present invention relates to a process for the preparation of an iron containing food additive that is prepared from plant matter comprising chlorophyll. The invention particularly relates to a simple and easy to carry out process starting with
10 plant matter, especially green leafy matter to prepare a food additive comprising iron which is in a highly bio-absorbable form. The invention therefore provides for nutrient supplementation in iron-deficient populations.

Background and Prior art

Iron is an important nutritional requirement of the human body. It is known that there is a large population of the world especially in developing countries, who suffer from iron
20 deficiency. This problem is more pronounced in people who have a vegetarian diet since the bio-absorption of iron is greater from a diet comprising meat and fish than it is from vegetables, cereals and pulses. Fortification of foods with 'edible' iron compounds or electrolytically reduced iron is one
25 of the possible approaches that is practised to overcome this problem. However, commonly used low cost fortificants tend to interact with and often discolour food and/or food ingredients. More importantly, the iron thus provided is not efficiently absorbed and utilised by the body.

30

The present inventors have developed a process starting with plant matter comprising chlorophyll to prepare a certain food additive that is rich in iron. Recognising the similarity in

the structure of chlorophyll with haeme it is believed that this product will be better bioabsorbed by the body thereby providing better utilisation of iron.

5 There are some reports of chlorophyll being treated with metals to prepare products for pharmaceutical and food uses. CN 1082049 (1994, Sibir) describes a process for preparing chlorophylline's alkaline salts of metal (Cu, Fe, Zn, Ge) which features the use of silkworm excrement and plant leaves.

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CN 1105028 (1995, Sibir) describes an organic or aqueous menstruum process to prepare coarse chlorophyllin alkali salt of metal (Cu, Fe, Zn, Ge) which is then refined and fine-filtered in sterile apparatus to obtain sterile non-pyrogen

15 chlorophyllin alkali salt of metal. The process includes direct vacuum drying method, menstruum crystalline method, spray drying method and vacuum freeze drying method.

CN 1263891 (2000, Huadong Normal Univ.) describes a process for
20 preparing zinc chlorophyllide which comprises the steps of washing and boiling leaves in water, crushing, extracting the chlorophyll in three stages with alcohol, saponification of the filtrate, reacting with Zinc sulphate in acidic medium, the residue treated with acetone and sodium hydroxide followed by
25 concentrating and drying the filtrate. The product is suitable for health medicines of anti-tumour, anti-pathological changes and as a food additive.

The prior art either consist of very complex processes which
30 involve treatment with organic solvent or use of complex machines. Also the processes have involved isolation of the chlorophyll from the plant matter before reacting with other metals. There is yet a need for a process which is simple to

carry out, can be done in aqueous media and involves minimal or no contact of the plant material with toxic/harmful chemicals.

It is thus an object of the present invention to provide for a
5 process to prepare a food additive comprising iron that is simple to carry out and does not require complex machinery and therefore can be prepared at a low cost.

It is another object of the invention to provide for a process
10 to prepare a food additive comprising iron that in addition to being prepared at a low cost is highly bioabsorbable by the human body.

It is yet another object of the present invention to provide
15 for a process to prepare a food additive comprising iron that is prepared at a low cost, is highly bioabsorbable in the human body and is prepared from plant matter without contacting the matter with toxic/harmful chemicals during the process thereby ensuring the preparation of a product that is highly safe for
20 human consumption.

Summary of the invention

25 The invention provides for a process for the preparation of a food additive rich in iron, the process comprising the steps of;

- (a) treating plant matter comprising chlorophyll with an aqueous solution of an acid, and simultaneously or
30 subsequently treating the plant matter with an iron containing compound,
- (b) neutralising the matter from step a) with an alkaline material to produce treated plant matter

- (c) separating water soluble material from the treated plant matter and
- (d) drying the treated plant matter to obtain a powder.

5 It is preferred that the acid treatment is carried out on the plant matter before the treatment with the iron compound is carried out.

It is particularly preferred that the plant matter is treated,
10 preferably by washing with water, to obtain a pH in the range of 4 to 7 before it is treated with the iron containing compound in step (a).

DETAILED DESCRIPTION OF THE INVENTION

15

The invention provides for a process for the preparation of a food additive that is rich in iron and is prepared from plant matter comprising chlorophyll. The plant matter is preferably any green part of a plant including leaves, roots and stem.

20 Autotrophic plants are especially preferred. Particularly preferred plant matter include leaves of vegetables for example leaves of spinach, cabbage, amaranth, radish, tomato, lettuce, fenugreek, beans and drumstick.

25 The plant matter comprising chlorophyll is first treated with an aqueous solution of an acid. Although no pretreatment is essential, it is preferred that the plant matter is treated to ensure removal of harmful chemicals and pathogenic microorganisms present on the surface of the plant matter. The
30 treatment preferably includes washing with water to remove extraneous matter like surface dust, soil, and pesticide residues. The pathogenic microorganisms may be removed by any known methods like treatment with hot water or steam. Blanching

is a commonly used method of ensuring kill of microorganisms. Blanching includes dipping in hot water followed by passing steam through the material. When carried out, blanching is usually done for a period of 15 to 45 minutes. It is preferable
5 that solvents in which chlorophyll is highly soluble are not used in any step of the process of the invention.

Although the acid treatment may be carried out on the plant matter in its original form, it is preferred that the matter is
10 mildly pulped to prepare a puree. Any known means of pulping may be used e.g. cutting, thrashing, chopping and known equipments like disintegrators, pulpers, or colloid mills may be used. Although the plant matter may be treated with any inorganic or organic acid, it is preferred that an inorganic
15 acid is used. Preferred inorganic acids are hydrochloric acid, sulphuric acid or nitric acid and highly preferred is hydrochloric acid. The preferred concentration of acids is in the range of 0.01 N to 5 N, more preferably in the range of 0.05 to 1 N. The treatment with acid is preferably carried out
20 for a period of 0.5 to 4 hours, more preferably 1 to 3 hours.

It is preferred that the plant matter is brought to a pH in the range of 4 to 7, especially 6 to 7, before treating with the iron containing compound. Preferred method of bringing the pH
25 to the range of 4 to 7 is by washing with water. Preferred iron containing compounds include organic and inorganic salts of iron. Highly preferred iron compounds are ferrous sulphate, ferrous chloride and ferrous nitrate. Although the iron compound may be added to the plant matter as a solid, it is
30 preferred that sufficient water is present in the system to ensure complete dissolution of the iron compound. Preferably, the iron compound is added as an aqueous solution with a concentration of 0.1 to 10%wt, preferably a concentration of

0.5 to 5%wt. The treatment with the iron compound is preferably carried out for a period of 2 to 24 hours, more preferably for a period of 5 to 15 hours, especially 8 to 12 hours, such as 10 hours. It is also possible that steps of the acid treatment and
5 treatment with the iron compound are carried out simultaneously or that the plant material is subsequently treated with the iron compound after the acid treatment. However, it is preferred that the treatment with the iron compound is carried out subsequent to the acid treatment. It is further preferred
10 that the treatment with the iron compound is carried out under a nitrogen atmosphere.

The plant matter is then optionally treated to ensure that water soluble materials are separated from the plant matter.
15 This is preferably done by washing with water. A sufficient amount of water is used for washing to ensure that most of the water soluble materials is removed. Preferably the iron compound added to the plant matter is removed to below detectable limits as determined by a colourimetric method. For
20 example, if the iron compound used is ferrous sulphate, the wash water is tested with potassium ferricyanide to ensure no colouration.

The plant matter is then treated with an alkali to raise the pH
25 in the range of 7 to 10, more preferably in the range of 8 to 9. Any alkaline material may be used, but carbonates, bicarbonates and hydroxides of alkali or alkaline earth metals are preferred. Highly preferred compound for neutralisation is sodium hydroxide.

30

The water and water soluble materials present in the treated plant matter are then removed to prepare a powder. The preferred method to do this is repeated washing with water and

filtration, followed by drying. The treated plant matter may be filtered using any known method of filtration including filtration by gravity, under vacuum, or by centrifugation. The treated plant matter may then be dried by any known method of
5 drying including sun drying, drying under a convective current of hot air, drying under vacuum either at high or freezing temperatures. Known drying equipments may be used including tray dryers, vacuum tray dryers, tumble dryers, flash/spin flash dryers, fluid bed dryers, or freeze dryers.

10

Without wishing to be bound by theory, it is believed that the magnesium in the porphyrin ring of the chlorophyll is effectively substituted and sufficiently well bound in the matrix by iron and this iron chlorophyll complex being
15 chemically similar to haeme, is more easily bioabsorbed into the human body. Thus the material prepared when used as a food additive would provide the desired iron supplementation in a simple, safe and cost effective manner. The oxidative states of the iron in the porphyrin ring was studied and was found to
20 be oxidation state 2 and oxidation state 3. This clearly shows the complexation of iron in the prepared powder.

The iron rich food additive of the present invention can be added into any conventional food and beverage products. The
25 food additive of the present invention has been added into products such as jams, sauces and biscuits etc.

The invention will now be demonstrated with the help of typical non-limiting example of the process according to the invention.
30 Further examples within the scope of the invention will be apparent to the person skilled in the art..

Example-1:

A quantity of 500 g of fresh leaves of spinach was taken. This was thoroughly washed and made into a puree using colloidal mill. Only a single run was given in the colloidal mill to prevent the puree from becoming very hot. This puree was blanched using a jacketed kettle with steam at 100°C for 15 to 20 minutes. About 250 ml of 0.2 N HCl was added to the puree and was stirred continuously for 2 hours. Sufficient water was added to the puree and stirred for sometime, and then the puree was filtered using a muslin cloth. This step was repeated sufficient number of times until it reached a pH of 5. About 250 ml of 2% ferrous sulphate solution was prepared and was added to the puree. The puree was then stirred for 10 hours. The puree was then washed with water. The filtrate was checked with 1% Potassium ferricyanide until there was no coloration to confirm the removal of all free ferrous ion. 250 ml of 1% sodium hydroxide in water was prepared and added to the puree. The puree was stirred continuously for 2 hours. The puree was washed with water until a pH of 9 was obtained. The water was then squeezed out and the wet matter was loaded onto a tray and was dried overnight at 80°C.

The magnesium and the iron content of the leafy matter at various stages of the process was measured using ICPOES method on a moisture free basis and the results are summarized in Table-1.

Table-1

Sample	Magnesium (%)	Iron (%)
Spinach leaves before step (a)	0.84	0.03

Puree after step (a)	0.04	0.03
Product after step (e)	0.01	1.08

The example demonstrates that product having high amount of iron can be prepared by the process of the invention and can serve as a food additive.

Claims

1. A process for the preparation of a food additive rich in iron, the process comprising the steps of;
 - (a) treating plant matter comprising chlorophyll with an aqueous solution of an acid, and simultaneously or subsequently treating the plant matter with an iron containing compound,
 - (b) neutralising the matter from step a) with an alkaline material to produce treated plant matter,
 - (c) separating water soluble material from the treated plant matter and
 - (d) drying the treated plant matter to obtain a powder.
2. A process as claimed in claim 1 wherein the plant matter comprising chlorophyll is treated with an aqueous solution of an acid prior to the treatment of the plant matter with an iron containing compound.
3. A process as claimed in either one of claims 1 or 2 wherein the plant matter comprising chlorophyll is any green part of a plant including leaves, roots and stem.
4. A process as claimed in any one of claims 1 to 3 wherein the plant matter is chosen from leaves of vegetables.
5. A process as claimed in claim 4, wherein the vegetables are chosen from spinach, cabbage, amaranth, radish, tomato, lettuce, fenugreek, beans and drumstick.

6. A process as claimed in any one of the preceding claims, wherein the plant matter is subjected to a step of blanching before treatment with an aqueous solution of an acid.
7. A process as claimed in claim 6 wherein the step of blanching is carried out for a period of 15 to 45 minutes.
8. A process as claimed in any one of the preceding claims wherein the plant matter is subjected to a step of pulping before treatment with an aqueous solution of an acid.
9. A process as claimed in any one of the preceding claims wherein the plant matter is treated with an aqueous solution of an inorganic acid.
10. A process as claimed in claim 9 wherein the inorganic acid is hydrochloric acid.
11. A process as claimed in any one of the preceding claims wherein the concentration of the acid is in the range of from 0.05 to 1 N.
12. A process as claimed in any one of the preceding claims wherein the treatment with an aqueous solution of an acid is carried out for a period of 0.5 to 4 hours.
13. A process as claimed in any one of the preceding claims wherein the plant matter is brought to a pH in the range of 4 to 7 before treating with the iron containing compound.

14. A process as claimed in any one of the preceding claims wherein the iron containing compound is chosen from ferrous sulphate, ferrous chloride or ferrous nitrate.
15. A process as claimed in any one of the preceding claims wherein the step of treating with the iron containing compound is carried out for a period of 5 to 15 hours.
16. A process as claimed in any one of the preceding claims wherein the treatment with the iron compound is carried out under a nitrogen atmosphere.
17. A process as claimed in any one of the preceding claims wherein water soluble material is separated from plant matter before the step of neutralisation.
18. A process as claimed in any one of the preceding claim wherein after the step of neutralising with an alkaline material, the treated matter has a pH in the range of 8 to 9.
19. A process as claimed in any one of the preceding claims wherein the alkaline material is sodium hydroxide.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/003635

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A23L1/304 A23L1/30		
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) IPC 7 A23L		
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 practical, search terms used) EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 804 536 A (FUKUDA ET AL) 14 February 1989 (1989-02-14) column 1, line 55 - column 2, line 12 column 2; example 2	1-19
A	----- PATENT ABSTRACTS OF JAPAN vol. 017, no. 167 (C-1043), 31 March 1993 (1993-03-31) & JP 04 327534 A (MITSUO NAMIKI; others: 01), 17 November 1992 (1992-11-17) abstract ----- -/--	1-19
☒ Further documents are listed in the continuation of box C.		
☒ Patent family members are listed in annex.		
° Special categories of cited documents :		
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed		
T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
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Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Inceisa, L

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE WPI Section Ch, Week 200023 Derwent Publications Ltd., London, GB; Class B04, AN 2000-264435 XP002333705 & JP 2000 069946 A (DAIWA SEIBUTSU KENKYUSHO KK) 7 March 2000 (2000-03-07) abstract -----	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

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